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ON

"STUDY ON HEMATOLOGICAL ABNORMALITIES IN VARIOUS STAGES OF CHRONIC KIDNEY DISEASE STAGE 3 TO 5"

SUBMITTED FOR M.D DEGREE EXAMINATION

BRANCH I

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CERTIFICATE

This is to certify that this dissertation entitled **"STUDY ON HAEMATOLOGICAL ABNORMALITIES IN VARIOUS STAGES OF CHRONIC KIDNEY DISEASE (stage 3-5)"** is the bonafide record work done by **Dr.V.PATTU SWARNA LATHA,** submitted as partial fulfillment for the requirements of M.D Degree Examinations, General Medicine (Branch I) to be held in APRIL 2014.

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This is to certify that the Institutional Ethical Committee of this College unanimously approves the Thesis /Dissertation/ Research Proposal submitted before this committee by Dr. V.PATTU SWARNA LATHA, **A POST GRADUATE IN MD MEDICINE**, Department of **MEDICINE** of Tirunelveli Medical College /Hospital, Tirunelveli titled **"STUDY OF HEMATOLOGICAL ABNORMALITIES IN VARIOUS STAGES OF CHRONIC KIDNEY DISEASE. (STAGE 3-5)"** registered by the IEC as 190/G.M./IEC/2012 dated. 11.07.2012. The Investigator is hereby advised to adhere to all the stipulated norms and conditions of this ethical committee.

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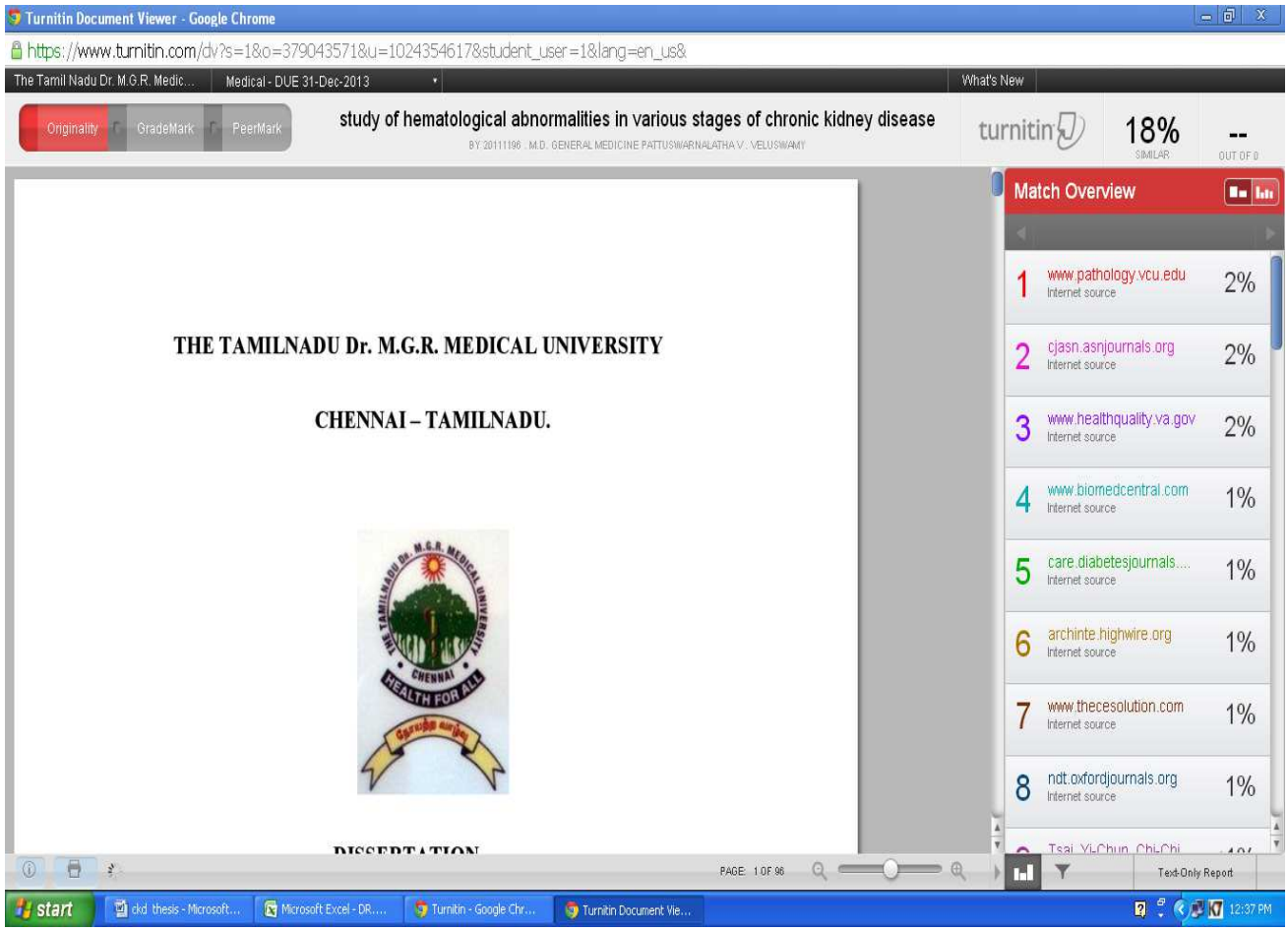
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ABSTRACT

Chronic kidney disease is one of the major health problems worldwide and a major cause of morbidity and mortality. CKD is diagnosed on the basis of presence of markers of kidney damage and kidney function. This study was done to assess the prevalence hematological abnormalities in CKD (stage3-5) and to assess their correlation among various etiologies of ckd(diabetes,chronic glomerulonephritis,hypertension).

The study was done in 150 cases diagnosed as CKD in the Department of Nephrology, IMCU, and in medical ward at tirunelveli Medical College Hospital. The diagnosis was based on estimated GFR level <60ml/mt/1.73M^2.

Total count, differential count, Hb, MCV, MCH, MCHC,WBC count, platelet count and peripheral smear examination, coagulation profile were done on all the patients and results were compared and correlated with each other.

Key Words :- Chronic Kidney Disease, Glomerular filtration rate, Anemia, Hemoglobin, White Blood Cell, leucocytes, Hemostasis, Platelet abnormalities.

INTRODUCTION

Chronic kidney disease has been a major public health problem. The hallmark of CKD is structural and/or functional damage of the glomeruli of the kidney. The most important result of renal damage are loss of renal function and cardiovascular disease leading to premature death. CKD is a progressive condition and with the progress of the disease the outcomes also progress to ultimately end up with kidney failure.

Earlier stages of CKD can be detected through laboratory testing. Accumulating evidence in the past 3 decades indicates that the identification of CKD in earlier stages can prevent its progression and delay the onset and progression of its outcomes.

There have been discrepancies worldwide regarding the definition, classification and laboratory testing of CKD resulting in lack of uniformity. In 2012, the National Kidney Foundation (NKF) and the Dialysis Outcomes Quality Initiative (DOQI) advisory board has approved the clinical practice guidelines to define the chronic kidney disease and stage CKD.[1]

The work group developed the following operational definition for chronic kidney disease (1) renal damage for ≥ 3 months as

defined by structural or functional abnormalities of the kidney, with or without decreased glomerular filtration rate (GFR), manifest by either pathological abnormalities or markers of kidney damage, or abnormalities in imaging tests.

(2) GFR less than 60ml/min/1.73m^2 for $\geq$ 3 months with or without kidney damage.

CKD is not a static condition. It tends to progress and worsen over time to ultimately end up with kidney failure because of the progress of the disease There are certain risk factors which alter the initiation and progress of CKD and its outcomes in an adverse manner.

<u>Risk factors which can alter the onset and progress of CKD and its outcomes in an adverse manner;</u>

- Age>65 yrs,
- Diabetes type 1 & 2,
- Hypertension.
- Atherosclerosis.
- Family history of renal disease
- Autoimmume disease.
- systemic infections.
- urinary tract infections/stones/obstruction.

- Medications (NSAIDS)
- Drug abusers (cocaine,heroin).
- Neoplasia.
- low birth weight, reduced kidney mass.

Identification of the presence of risk factors and institution of appropriate therapeutic interventions in the early stages of CKD are effective in slowing the onset and progression of CKD.

CKD is also associated with certain complications the most important of which are anemia, hypertension, neuropathy and nutritional imbalance.

Anemia is a common co-morbid finding in kidney failure patients. The patients with anemia in chronic kidney disease most frequently present with complaints pertaining to compromised renal functions. A patient with chronic kidney disease is said to besuffering from anemia if the hemoglobin concentration is <13g/dl in adult males and if the Hb is <12g/dl in adult females[2.]

This level of Hb are reached when the GFR is reduced to <30% of the normal value which corresponds to the serum creatinine level of 2-4mg/dl . Irrespective of the type of CKD anemia can develop. Only in

two conditions viz. polycystic kidney disease and hypertension usually anemia does not develop [3.]

The different morphological types of anemia occurs in CKD. Normocytic normochromic type which is the most common followed by normocytic hypochromic type and microcytic hypochromic type which is the least common. While anemia being a most common finding in CKD patients, leucopenia and thrombocytopenia worsens as ckd stage progresses. This is probably because of inhibition of erythropoiesis, granulopoiesis and megakaryopoiesis, by the uremic inhibitors. Morphology of white blood cells appears within normal limits But clinically the patients present with an increased incidence of infections in uremia due to abnormal chemotactic function and defective receptor regulation[4].

Also qualitative platelet defect occurs. There is decreased platelet adhesiveness and aggregation in response to adenosine diphosphate and decreased release of platelet factor III, due to the accumulation of toxic metabolites in uremia [5].

The present study is an attempt at comprehensive review of red blood cells (RBCs), leucocytes, platelets & coagulation profile and to assess their significance in CKD.

OBJECTIVES

(1) To study the prevalence of hematological abnormalities in various stages of chronic kidney diseases(stage3-5).

(2) Their correlation among various etiologies of CKD (DM/chronic glomerulonephritis/hypertension)

REVIEW OF LITERATURE

General:

Chronic kidney disease is one among the significant reasons for morbidity and mortality world wide[6]. Kidney diseases is ranked - 3rd amongst life threatening disease in India, after malignancy and heart disease. About 100,000 persons go into terminal kidney failure every year.

Chronic kidney disease is a condition characterized by progressive and irreversible deterioration of renal function due to slow destruction of renal parenchyma, eventually terminating in death when sufficient numbers of nephrons have been damaged.

The clinician caring for a patient with CKD must first differentiate whether the renal insufficiency is acute or chronic.

The initial differential diagnostic approach to chronic renal insufficiency consists of ascertaining whether the individual has glomerular leison or interstitial leison or vascular pathology on the basis of careful history taking, urine analysis and measurement of 24 hour protein excretion. Further refinement of diagnostic considerations requires imaging of urinary system, serologic studies, and renal biopsy.

Management begins with identification and rectification of all acute reversible causes of renal insufficiency in cases with CKD. Once the therapeutic options are in place, it is neccesary to anticipate and treat varied manifestations of CKD .

DEFINITION OF CHRONIC KIDNEY DISEASE :

1.The work group of DOQI advisory board included situations that affect the kidney with ability to cause either progressive loss of renal function and complications resulting from decreased kidney function.

CKD was thus defined as presence of kidney damage or reduced level of kidney function for three months or more irrespective of the diagnosis.

Chronic kidney disease is defined as

(1) Kidney damage [7] for 3 months or more, as defined by structural or functional abnormalities of the kidney, with or without reduced GFR, manifested by either:

(a) Pathological abnormalities detected by histopathological studies; or

(b)Markers of kidney damage, including abnormalities in the composition of blood or urine, or abnormalities in imaging studies.

(2) GFR less than 60ml / min /1.73m2 for 3 months or more, with or without kidney damage.

The detection and classification of CKD is dependant on the evaluation of markers of renal function and markers of renal damage.

The best accepted measure of kidney function is GFR. Kidney damage may or may not be associated with decreased kidney function. Cases with renal damage are at an elevated risk of loss of renal function. The explanation for GFR to be normal in spite of kidney damage is as follows.GFR is the product of the number of nephrons [8].

So, the decline in GFR depends upon the number of nephrons and the extent to which each nephron is affected. In the initial stages of kidney damage, when only a few glomeruli are damaged, GFR is maintained because of adaptive elevations in glomerular capillary blood flow and pressure and due to an increased tubular secretion of metabolites in response to reduced ultra filtration coefficient and decreased number of nephrons [9]. But in later stages, when more and more glomeruli are damaged, it results in a decline in GFR and the decline progresses as the kidney damage worsens, to end with kidney failure.

The second criterion for the diagnosis of CKD is GFR <60ml / min / 1.73m^2 with or without kidney damage. GFR could be reduced in spite of absence of kidney damage in infants and older adults, vegetarians, single kidney, hypovolemia, heart failure, cirrhosis[10]. The rationale for this level of GFR is that reduction in kidney function to this level represents loss of half or more of the adult level of normal kidney function[11] and that range of normal GFR levels vary widely, as explained later.

CONFIRMATION OF CHRONICITY OF THE KIDNEY DISEASE.

This could be achieved through the following:

a. History: A long history of renal disease suggests chronicity. absent previous history suggests acute renal failure.

b. Kidney size as detected by ultrasonography: A small echogenic kidneys < 8 cm favours the diagnosis of chronic kidney disease, while a normal sized kidneys is more in favour of acute renal failure.

There are some conditions of chronic kidney disease in which kidney size is within normal limits or even increased (>13 cm) these are:

- Diabetic nephropathy.
- Renal amyloidosis

- Infiltration (leukaemia, lymphoma, sarcoidosis).
- polycystic kidney disease.
- HIV nephropathy.
- Obstructive uropathy.
- Bilateral staghorn stone.

(c). Magnitude of the increase in serum creatinine in relation to the presenting symptoms High serum creatinine with minimal symptoms favours chronic kidney disease, while relatively low serum creatinine with severe symptoms is in favour of acute renal disease.

(d) hyperphosphataemia and osteodystrophy : are present more with chronic cases.

(e) Anaemia is more with chronic cases.

Staging of CKD:

The two important outcomes of CKD are loss of kidney function and development of cardiovascular complications. These depend upon the severity of CKD. So it is important to stage CKD based on its severity and this is done on the basis of levels of GFR, as follows.

Stages of chronic kidney disease:[12]

STAGE	DESCRIPTION	GFR(ML/MIN/1.73 M^2)
1	Kidney damage with normal or increased GFR	$\geq$ 90
2	Kidney damage with a mild decrease in GFR	60 - 89
3	Moderate decrease in GFR	30 - 59
4	Severe decrease in GFR	15 - 30
5	Kidney failure	< 15

In CKD stage 3-5, there occurs continous and permanent reduction in nephron number.

The distressing term end-stage renal disease (ESRD) is that stage of CKD where there occurs accumulation of , fluid, toxins and electrolytes that are normally excreted by the kidneys resulting in the uremic syndrome, that culminates in death unless the toxins are removed by renal replacement therapy, such as dialysis or kidney transplantation. End-stage renal disease corresponds to stage 5 CKD.

EVALUATION OF MARKERS OF RENAL FUNCTION

(A)Glomerular filtration rate: It has been the best accepted measure of renal function, both in health and disease. Certain aspects has to be considered while interpreting GFR. GFR is calibrated

for body size. In young adult males, the normal mean GFR is 120-130ml/min/1.73m^2. Children reach adult values by approximately 2 years. After approximately age 20-30years, GFR declines with age, fall equals to 1ml/min/1.73m^2 every year. So when one becomes 70, GFR is 70ml/min/1.73m^2. In women GFR is 8% lower at all ages. GFR increases in pregnancy and after high protein diet. It decreases after low protein diet and antihypertensive agents [8,13]. For this reason, definition of CKD is based solely on GFR only when GFR is less than 60ml/min/1.73m^2. If GFR is more than 60ml/min/1.73m^2, a diagnosis of CKD is made only in the presence of kidney damage[14] .

ESTIMATION OF GFR :

The Egfr has been accepted as best measure of kidney function in patients with CKD and has replaced serum creatinine measurement as the diagnostic test of choice for CKD.

GFR can be directly measured using a variety of different assays, but they are not feasible for widespread use in the clinical setting. A variety of different prediction equations have been developed including the MDRD and Cockroft-Gault Formulas.

Estimates of GFR may be unreliable at the extremes of age, muscle mass and weight, and at eGFR levels above 60 ml/min/1.73m^2,

but it is a reasonably accurate measure of true GFR in most patients with moderate or more severe CKD . Both MDRD and the Cockroft-Gault equations are acceptable for use in the clinical setting.

These equations also consider age, sex, body size and race because of the variations in GFR and creatinine metabolism with respect to these factors.

The equations used are:

(1)EQUATION FROM THE MODIFICATION OF DIET IN RENAL DISEASE STUDY.

Estimated GFR (mL/min per 1.73 m^2) = 1.86 x $(P_{Cr})^{-1.154}$ x $(age)^{-0.203}$

Multiply by 0.742 for women and Multiply by 1.21 for African Americans.

(2) COCKCROFT-GAULT EQUATION

$$\text{Estimated creatinine clearance (ml/min)} = \frac{(140-\text{age}) \text{x body weight (kg)}}{72 \text{ x PCr (mg/dL)}}$$

Multiply by 0.85 for women.

Pcr - Serum Creatinine concentration

(3)Schwartz and Counahan-Barratt formulae in children. Serum creatinine levels should be calibrated to the levels recommended by those laboratories which have formulated these equations[15].

In patients with low muscle mass until creatinine clearance has declined to less than half of normal , serum creatinine will not rise above the normal range. the serum creatinine concentration doubles approximately, for every 50 percent reduction in GFR. It has been identified that since serum creatinine is determined both by kidney function and muscle mass, patients with normal serum creatinine and low muscle mass may have significant impairment of kidney function. Patients with a serum creatinine level above the normal range but under 2.0 mg/dL have significant kidney disease but are less likely to have electrolyte disturbances, anemia, or bone disease than those patients with creatinine level of >2.0 mg/dL.

(4) CREATININE CLEARANCE: using timed urine samples is cumbersome and it is not anyway superior to the GFR estimates that are provided by prediction equations. However, it is useful for estimation of GFR in patients who consume only vegetarian food, in malnutrition individuals, or after amputation and muscle wasting[16].

(5) INULIN CLEARANCE had been accepted widely as the gold standard procedure , but assessment of inulin clearance necessitates an intravenous infusion and timed urine collection over a period of several hours making the procedure cumbersome.

(B)SERUM CREATININE:

This is not an accurate measure of level of renal function. It is dependant on individual being in steady state and the ability to estimate the average rate of production of creatinine. Estimates will be unreliable if the level of GFR is changing, if muscle mass is unusually high or low or if dietary creatinine intake is unusually high or low[18]. Changes in serum creatinine levels do not correlate linearly with changes in GFR. In patients with early renal impairment a small increment in serum creatinine levels may reflect a large percent decrease in GFR[19]. SCr values corresponding to GFR ranges overlap and therefore cut off values for staging of CKD are arbitrary [15]. In spite of these problems, SCr level is used as a gross indicator of kidney function widely in clinical medicine. The overall mean SCr value for persons aged 12 years and older is 1.16mg/dl in men and 0.96mg/dl in women. At a SCr level of 2.0mg/dl, mean GFR is less than 50% of normal [19.]

EVALUATION OF MARKERS OF KIDNEY DAMAGE:

(1) Proteinuria:

Proteinuria is a very early and sensitive marker of kidney damage. It is manifested at the very start of the kidney damage unlike decline in GFR, which is manifested only after substantial kidney damage[20].

It is a potential independent risk factor for progression of renal disease and independent cardiovascular risk factor. Proteinuria is assessed using a conventional dipstick method. A first morning urine specimen is preferred, but random urine specimens are also acceptable. Proteinuria accelerates the rate of decline of GFR in hypertensive, diabetic, as well as in non-diabetic individuals.

Proteinuria includes increased excretion of total urine protein, albuminuria, increased excretion of other specific proteins. Albuminuria refers to only increased urine albumin excretion. Microalbuminuria refers to very small amounts of albumin. Albuminuria is a more specific indicator of kidney damage in terms of glomeruli because proteinuria includes proteins from urinary tract and other low molecular weight proteins filtered by normal glomeruli[21].

Defined cut off values in CKD [22].

- Proteinuria of more than 300mg/day.
- albuminuria of more than 300mg/day.
- microalbuminuria of 30-300mg/day.

While assessing proteinuria/albuminuria, orthostatic proteinuria / albuminuria should be ruled out by comparing a measurement of protein excretion in a overnight recumbent urine collection to a daytime upright

urine collection. Persistent proteinuria/albuminuria should be assessed [15]. Sustained proteinuria >1–2 g/24 h is commonly associated with glomerular disease. proteinuria will not be identified until patients become edematous or notice foaming of urine on voiding. Sustained proteinuria has to be distinguished from benign proteinuria in the normal population,which is nonsustained, generally <1 g/24 h, and is sometimes called functional or transient proteinuria. Fever, exercise, obesity, sleep apnea, emotional stress, and congestive heart failure can explain transient proteinuria. Proteinuria only seen with upright posture is called as orthostatic proteinuria and has a benign prognosis. Isolated proteinuria sustained over multiple visits is found in diabetic nephropathy, membranoproliferative glomerulonephritis , and FSGS. Proteinuria in most adults with glomerular disease is nonselective, containing albumin and a mixture of other serum proteins[23.]

(2) Urine sediment examination: Presence of RBCs-normal and dysmorphic and WBCs indicates CKD. But they are not sensitive and specific. They can be absent in CKD and be present in pathology of tubules, interstitium and urinary tract[24].

(3)Imaging studies: They suggest intrinsic kidney disease[24]. Renal ultrasonagraphy helps to establish the diagnosis and prognosis by

documenting the size of the kidneys. Normal size indicates kidney disease that may be amenable to medical treatment.

Classification of CKD by pathology and etiology[25]

Pathology	Etiology	Prevalence
Diabetic glomerulosclerosis	Diabetes Mellitus	33%
Vascular diseases	Hypertension, renal artery stenosis, HUS	21%
Glomerular diseases	SLE, vasculitis, amyloidosis, drugs, HIV	19%
Cystic diseases	Autosomal dominant or recessive	6%
Tubulointerstitial diseases	Infection, stones, NSAIDs, antibiotics	4%

HUS-Hemolytic uremic syndrome, SLE-Systemic lupus erythematoses, HIV-Human immuno deficiency virus, NSAIDs-Non steroidal anti inflammatory drugs.

Diabetes mellitus has been the commonest etiology for CKD throughout the world and in India [26, 27]. From the table it is evident that apart from glomerular diseases, tubulointerstitial diseases, vascular diseases and cystic diseases contribute to CKD. But because the

explained markers are specific for glomerular damage and not for tubulointerstitial and vascular damage, criteria are not standardized for these conditions. However the diagnosis of CKD in these conditions is also based on GFR and SCr. GFR might be normal in tubulointerstial diseases where glomeruli is spared in the disease process, these cases might go undiagnosed as CKD.

The diagnosis of underlying disease is by serology, ultrasonography and renal biopsy. The diagnosis is important as, when the disease progresses it worsens the kidney damage. Appropriate therapeutic intervention and control of the underlying disease will prevent the progress of CKD [26,27].

As already stated, CKD is not a static condition. It tends to progress and worsen over time to ultimately end up with kidney failure. There are many risk factors, which influence this progression and outcomes of CKD in an adverse manner**.**

FACTORS WHICH INFLUENCE THE PROGRESSION OF CKD:

- Hypertension.
- diabetes mellitus.
- African ancestry

- older age.
- Autoimmune diseases.
- Family history of renal problem.
- Previous history of acute kidney injury.
- presence of any proteinuria and abnormal urinary sediment.
- structural abnormalities in the urinary tract.
- Genetic predisposition-allelic versions of the APOL1 gene. (focal segmental glomerulosclerosis).[28]

The extent to which these factors can affect the outcomes of kidney damage depends on the severity of the disease and rate of progression[29]. Identification of the presence of risk factors and instituting appropriate therapeutic intervention in the early stages of CKD are effective in slowing the progression of CKD.

Clinical presentation of CKD

The clinical presentation of these patients will be representative of kidney damage, loss of kidney function and of the causative disease entity. The signs and symptoms are grouped as uremic syndrome[30] characterized by circulating uremic toxins. It is associated with complications in virtually all organs.

Stages 1 and 2 CKD has not been associated with any symptoms, there may be symptoms from the underlying renal disease itself, such as edema in patients with nephrotic syndrome and signs and symptoms of hypertension secondary to the renal parenchymal disease in individuals even with well-preserved GFR. When GFR declines to stages 3 and 4, all clinical and laboratory complications in CKD become prominent. any organ system could be affected. Anemia usually presents with easy fatiguability, poor appetite and progressive malnutrition, abnormalities in mineral calcium, phosphorus metabolism and hormones, such as $1,25(OH)_2D3$ (calcitriol), parathyroid harmone (PTH), and fibroblast growth factor 23 (FGF-23) are altered; as well are abnormalities in electrolytes, water, and acid-base homeostasis.

Most individuals , especially elderly aged, will have eGFR values that are compatible with stage 2 or 3 CKD and majority of these patients will show no further decline of renal function. when the patient progresses to stage 5 CKD, toxins begin to accumulate , and the patients start experiencing a marked disturbance in their activities of daily living, well-being, nutritional status, and water and electrolyte homeostasis, resulting in the uremic syndrome. Which will eventually lead to death unless renal replacement therapy like dialysis or transplantation has been instituted.

When a patient presents with signs and symptoms of CKD, the evaluation proceeds as follows:

1) Confirmation of the presence of CKD by assessing markers of kidney injury and markers of reduced kidney function.
2) Identification of the underlying disease leading to CKD and employing appropriate therapeutic intervention so as to prevent the disease from damaging the kidney further.
3) Identification of the presence of risk factors and employing measures to check them, to prevent the adverse outcomes of CKD.
4) Employment of treatment strategies for CKD itself, depending upon the severity of CKD. Early stages might not require intervention but kidney failure will need some form of replacement therapy.
5) Identification of complications as a part of uremic syndrome and undertaking appropriate measures to correct them.
6) Constant evaluation of the progress of the disease so as to have an idea as to when approximately the patient will have kidney failure. This will help plan replacement therapies.

Some patients with early stages of CKD are asymptomatic. If any individual is known to suffer from any of the diseases known

to cause CKD, he should be under constant and periodical screening . The disease should be maintained at adequate control so as to prevent the onset of CKD.

Anemia of chronic kidney disease

Anemia is both a complication of CKD as a part of uremic syndrome as well as risk factor, which influences the adverse outcomes of CKD. So evaluation and management of anemia is of prime importance to prevent the progress of CKD and for the general well being of the patient. The erythropoietic system is affected by numerous ways, with the primary clinical outcome, being reduced erythrocyte mass and anemia. This complication of renal failure was first observed by Richard Bright in 1836.[31]

Anemia in CKD being one of the earliest signs of renal dysfunction, but because of its insidious onset it usually goes undetected . Anemia usually develops gradually when kidney function declines and the GFR has droped to 70 mL/min in males and 50 mL/min in female patients.

Hemoglobin measurement have been the preferred laboratory investigation for diagnosing anemia. Hematocrit can also be used ,

but it has been subject to measurement error because of sample storage and differences in analytic equipments.

Definition and identification of anemia in CKD: Anemia can be diagnosed in adults and children above 15 years with CKD when the Hb concentration is less than 13.0 g/dl (130 g/l) in males and less than 12.0 g/dl (120 g/l) in females.

Definition of anemia, in this study anemia was defined using The World Health Organization (WHO) criteria, hemoglobin concentration lower than 13.0 g/dl in men and lower than 12.0g/dl in non pregnant women.

- hemoglobin10 - 12.9 g/dl for men and 10 -11.9 g/dl for women was used to define mild anemia.
- hemoglobin 7 - 9.9 g/dl for both genders defined moderate anemia
- hemoglobin < 7 g/dl for both genders defined severe anemia[33].

Evaluation of anemia in people with CKD:

For identification of anemia in people with CKD, measure Hb concentration,

- When indicated clinically in people with GFR more than 60 ml/min/1.73 m^2(stage 1 and 2).

- Once in a year in people with GFR 30–59 ml/min/1.73 m^2 (stage 3 CKD).
- twice per year in people with GFR less than 30 ml/min/1.73m^2 (stage 4 and 5 CKD).

IMPACT OF ANEMIA IN CKD PATIENTS:

1. Anemia in CKD patients have been associated with cardiovascular complications. Anaemia have been independently associated with the development of left ventricular hypertrophy (LVH), which is an independent predictor of subsequent cardiac morbidity and mortality.
2. The presence of anemia have been associated with a greater rate of hospitalizations and mortality.
3. CKD patients suffering from anemia usually have an impaired quality of life, exercise capacity, cognitive and sexual function .
4. Anemia in CKD patients also have increased blood transfusion requirements.

The causes of anemia can be broadly categorized as follows [34, 35, 36]

1. DECREASED BONE MARROW SYNTHESIS:

a) bone marrow suppression as a part of uremic syndrome [32,35,36]

b) Decreased Erythropoietin production [34]

c) Bone marrow fibrosis due to increased parathyroid hormone [39]

d) Inflammatory cytokines: Increased levels of inflammatory cytokines has been found in patients with CKD, because of inflammation in the glomeruli as a part of the disease process causing CKD or because of concomitant infections in CKD. Inflammatory cytokines , inhibit the production of EPO and they render erythroid cells insensitive to the action of EPO [47] leading to a normocytic normochromic blood picture. Inflammation upregulates hepcidin, a protein synthesized in liver that reduces gut iron absorption and interferes with iron release from the reticuloendothelial system to the developing erythron.

2. SHORTENED RED CELL SURVIVAL:

a) As a part of uremic syndrome RBC survival is decreased in uremic patient's in proportion to the blood urea nitrogen concentration and, it improves significantly after intensive hemodialysis. Increased expression of phosphatidylserine occurs on the outer cell surface of red blood cells due to uremic environment in plasma. This enhances the recognition of damaged red blood cells by macrophage, leading to their subsequent destruction and decreased survival[38]

b) Increased osmotic fragility of RBCs due to increased Parathyriod harmone[37]

c) Hemolysis due to concomitant infections.

d) Hemolysis due to microangiopathy- primary disease manifestation.

e) **Dialysis** itself can contribute to the anemia. Iron deficiency can result from unavoidable dialyzer blood loss, frequent blood sampling and clotted dialysis membranes. Hemolysis will occur if there are problems with the dialysate such as temperature problems, contamination with copper, aluminum , chloramine, chlorine, or fluoride. Folic acid, a water soluble vitamin necessary for normal red blood cell synthesis, is dialyzable. usually, dialysis patients has to be given ,oral supplementation with folic acid in case their normal diet does not meet the sufficient folate required to keep up with its loss through dialysis.

(3) Hemodilution due to fluid overload.

(4) Iron deficiency [34]

The causes are listed below :

- Reduced iron absorption due to uremic syndrome
- Loss of iron and RBCs due to bleeding tendencies in uremic syndrome

- loss of transferrin in urine as a part of proteinuria, causing impaired iron transport
- Dialysis related loss of RBCs and iron
- Blood loss due to frequent blood sampling.

(5) Folate and vitamin B12 deficiency [34]

(6) Electrolyte imbalances [40]

(7) Aluminium toxicity [34]

(8) Underlying hemoglobinopathies [40]

(9) Comorbid conditions, pregnancy, HIV, hypo/hyperthyroidism, autoimmune disease.

(10) Bleeding tendencies.

(11) Hypersplenism especially in multiple transfused patient.

(12) Immunosuppressive drug especially mycophenolate mofetil,tacrolimus, sirolimus intake is associated with low hematocrit.

Anemia in azotemia as a part of uremic syndrome [35, 36].

Uremic syndrome is characterized by circulating toxins which accumulate in blood due to decreased excretion by the diseased kidney [35,32, 36,38] These toxins have deleterious effects on many organ systems.

The circulating toxins are:

a) Toxic products of protein metabolism-urea, guanidino containing compounds[37].
b) Advanced oxidation products
c) Products of bacterial metabolism
d) Homocysteine
e) Electrolytes [37]
f) Metabolic acidosis [37]
g) Polyamines -spermine and spermidine[36].

These circulating toxins, specifically suppress the erythroid series in the bone marrow. There is a decrease in the erythroid mitoses . The arrest is in the erythroblastic stage, resulting in the formation of very few younger forms of the series. These toxins inhibit erythroid cells in a non specific way.

They do render these cells insensitive to the action of erythropoietin, a hormone secreted by kidney which is responsible for the maintenance of normal erythropoiesis[32]. Erythropoietin is the hormone which has been the major humoral regulator of red cell synthesis and helps in maintaining the RBC viability , by retarding the cleavage of DNA that occurs normally in colony forming unit-erythroid. In the absence of EPO, DNA cleavage is rapid and leads to

cell death. This probably explains the selective suppression of erythroid cells by uremic toxins. This is reflected in the peripheral blood picture as a normocytic normochromic anemia.

This is the most common type of anemia in CKD. The Hb, RBC count and Hct decrease but the mean corpuscular volume (MCV), the mean corpuscular hemoglobin content and concentration (MCH and MCHC) respectively remain unaltered.

The anemia of CKD have a number of adverse pathophysiologic consequences, including reduced oxygen delivery to tissues and utilization, increased cardiac output, ventricular dilation and hypertrophy. Clinically presents with easy fatiguability and reduced exercise tolerance, angina, cardiac failure, diminished intelligence and mental acuity, and impaired host defenses against infection. Also, anemia may play a role in growth retardation in children with CKD. While many studies in CKD patients have found that anemia and resistance to exogenous EPO are associated with a poor prognosis, the relative contribution to a poor outcome is by low hematocrit itself.

Abnormalities of leukocytes

The abnormalities with respect to leukocytes are qualitative, they being functionally defective. Granulocytes show impaired

migration and abnormal chemotactic activity. Macrophage Fcγ receptor is impaired[4]. Functional effects on cell mediated immunity has been demonstrated by depressed delayed type hypersensitivity ,this leads to increased incidence of infections in spite of increased levels of leukocytes. also reduction in natural killer cells occur There may be lymphopenia. The Na+kATPase is defective in leukocyte membrane leading to loss of leukocytes[41] .Inflammation is a major pathogenetic factor in chronic kidney disease. Inflammation mediates influx of monocytes , macrophage proliferation and matrix expansion, resulting in glomerulosclerosis and tubulointerstitial injury which will further exacerbate renal injuries. white blood cell count has been a traditional marker of inflammation and infection responses, and former studies have showed that their have been a significant association of WBC count and poor outcome in dialysis patients. In Atherosclerosis Risks in Communities (ARIC) study, it has been showed that increased WBC count is correlated with greater risk for renal function deteriotation , but Kovesdy et.al study revealed the opposite observation.

<u>Inflammation in chronic kidney disease patients is due to:</u>

- Poor appetite leading to malnutrition.
- Infection of dialysis vascular access.

- Periodontal infections.
- Foot ulcers.
- Anemia.
- Uremic toxins.
- A transplanted kidney that no longer works

There is a relation between malnutrition and chronic inflammation in patients with ckd. Poor appetite leads to low calorie and protein intake and over a period of time protein energy malnutrition results. ultimately culminating in weight loss and muscle wasting. inflammation interferes with the body's ability to make new proteins, leading to muscle wasting.In a person with inflammation, cytokines are released. Normally cytokines help to defence against infection. but, cytokines also reduce the appetite and slow down gastric emptying. If chronic inflammation is not treated, cytokines contribute to chronic low albumin levels and muscle wasting . hypoalbuminemia is used as a marker for malnutrition and inflammation in kidney disease patients . they have poor quality of life, low hemoglobin levels and increased hospitalizations.

Patients with CKD have more spikes in leukocyte counts than their non-CKD counterparts. These spikes are between 1.5- and 3.0-fold higher in those with CKD. Spikes in absolute and percent eosinophil

count and percent granulocyte and percent monocyte counts are independent risk factors for the combined endpoint of all-cause mortality and ESRD. Granulocyte and monocyte spikes were independently associated with ESRD and death in this population. Thus, inflammatory events , among patients with CKD have significant prognostic importance. Inflammatory events were associated with an increased risk of ESRD and death. These inflammatory events also includes those confined to the kidney, which may accelerate the decline in renal function. Many recent studies , have increasingly reported prominent clusters of inflammatory cells, particularly macrophages, in renal biopsy specimens from patients with diabetic nephropathy . Peritubular macrophages and granulocytes were inversely correlated with estimated GFR at the time of biopsy. However, clinically it is perhaps more likely that these inflammatory events also included inflammatory illnesses such as pneumonia, bacteremia, and urinary tract infections. Such illnesses are associated with volume depletion and nephrotoxin exposure and perhaps induce an inflammatory response in the kidney, factors that may have caused an accelerated time to ESRD and/or death. Others have reported an increased risk of mortality with neutrophil count among hemodialysis patients.

Spikes in eosinophil counts were also associated with ESRD/death outcomes. eosinophilic spikes may be due to subclinical atheroembolic events or allergic responses to environmental antigens or drug exposures. Another suggested possibility is that of deficiency or resistance to cortisol. Studies have described that their is relationship between stress and hypercortisolemia and eosinopenia. In those with corticosteroid deficiency, eosinophilia may be seen. Patients with CKD may have resistance to cortisol and eosinophilia. In a recent cross-sectional study, eosinophilia was associated with albuminuria . Kovesdy et al., who have reported among CKD veterans not on dialysis an inverse relationship between lymphocyte count and all-cause mortality. Similarly, low lymphocyte counts are associated with mortality among hemodialysis patients.

Effect of dialysis on leukocytes: Hemodialysis leads to transient neutropenia within 2 hours after initiation of dialysis due to the sequestration of granulocytes in the dialysis apparatus. It also leads to loss of inflammatory cytokines in the permeable biocompatible membrane resulting in impaired immunity.

Continous Ambulatory Peritoneal Dialysis suppresses inflammatory cells and has cytotoxic effect on mesothelial cells leading to impaired

cellular defense mechanism against bacterial peritonitis. The dialysis fluids are the culprits[42].

PLATELETS AND HEMOSTASIS IN CKD [6]

Patients in advanced stages of CKD may have a reduced platelet factor III activity, abnormal platelet aggregation and adhesiveness, and impaired prothrombin consumption and prolonged bleeding time. Clinical presentations include an increased bleeding tendency and bruising, menorrhagia, haematemesis and malena from spontaneous GI bleeding, prolonged bleeding from surgical incisions.

Also, CKD patients have a increased tendency for thromboembolism, usually if they have renal problem that includes nephrotic-range proteinuria which results in hypoalbuminemia and renal loss of anticoagulant factors leading to a thrombophilic state.

ABNORMALITIES OF PLATELETS:

In CKD, impaired erythropoietin secretion leads to a decrease in platelet count. Receptors for erythropoietin has been detected in megakaryocytes and because of extensive homology between erythropoietin and thrombopoietin, erythropoietin also act as the major humoral regulator of platelet mass. Erythropoietin potentiates the action of megakaryocyte colony stimulating factors, acetlyhydroase (PAF-

AH) and paraoxonase (PON1)[43]. In patients with chronic kidney disease treated with erythropoietin, a small increase in platelet count, averaging approximately 30,000 per microliter has been noted. Platelet dysfunction is the most consistent and important feature. Dialyzable substances like urea, creatinine, guanidinosuccinic acid, phenol which accumalates in uremia causes impairment of platelet aggregation. But dialysis does not correct prolonged BT. Bleeding diathesis usually disappears after renal transplantation**.**

Also following qualitative platelet abnormalities have been observed:

- Decreased platelet factor III release.
- Decreased platelet adhesiveness and aggregation in response to adenosine di phosphate.
- Storage pool defect and decreased dense granule content.
- Defective arachidonic acid metabolism and decreased release of endogenous arachidonic acid from platelet phospholipids leading to decreased production of thromboxane A2 which is the main abnormality.
- Decreased IIb-IIIa receptors on platelets[5].
- Reduced sensitivity to platelet agonists.
- Abnormal expression of platelet glycoproteins and
- Depressed prostaglandin metabolism.

CAUSES FOR PLATELET DYSFUNCTION:

- Uremic toxins:- Inhibitors of low molecular weight which are guanidine succinic acid, phenolic acids, urea or a combination of these.
- Low hemoglobin levels.
- Increased nitric oxide production.
- Abnormalities in Von Willebrand factor.
- Use of medications like non-steroidal anti-inflammatory drugs, aspirin, and β-lactam antibiotics.

HEMOSTATIC ABNORMALITIES IN CKD:[44]

The balance between coagulation and anti-coagulation is altered in chronic kidney disease. Initial stages of chronic kidney disease are usually associated with prothrombotic tendencies , whereas in its more later stage patients also suffer from a bleeding tendencies.

Bleeding tendencies:

Patients with CKD have bleeding tendency which is manifested by ecchymoses, purpurae, epitaxis, prolonged bleeding from venipunture sites. In his Epistola Anatomica-medica XLI, the Italian anatomist and pathologist Giambattisa Morgagni (1682-1771) described a woman with severe bleeding (epistaxis and hematemesis) who had the odour of urine on her breath. This is probably the first description of association of

uremia and bleeding tendency. Because of the introduction of dialysis, bleeding in uremia is more easily controlled. However the underlying bleeding diathesis remains.

Following are reasons;

- Thrombocytopathy.
- Thrombocytopenia.
- Anemia.
- Increased activated protein c.
- Defective structure and distribution of von Willebrand factor leading to poor platelet aggregation.
- Deficiency of factors II, VI, IX, XII, XIII as they are lost in urine.
- Dysfibrinogenemia are the factors which lead to a bleeding tendency in uremia.

Bleeding time being a universal test which is measured by making a small incision on the upper arm, earlobe, finger, or thigh and normally is between 1 and 7 minutes. The characteristic laboratory finding is a prolonged bleeding time longer than 8 minutes. BT is the best predictor of uraemic bleeding, but suffers from poor reproducibility and

accuracy[44]. Alternative in vitro test to replicate BT has not been identified.

Treatment regimens like cryoprecipitate and DDAVP are used in emergent situations.when immediate action is unnecessary conjugated oestrogens and initiation of dialysis are appropriate.

Thrombotic tendencies:

In addition to increased bleeding risk, a variety of thrombotic complications has been observed in patients with chronic renal failure, including coronary artery disease, cerebrovascular disease, peripheral vascular disease and cardiac failure. Already in mild to moderate chronic kidney disease an increased risk for cardiovascular disease and slightly higher mortality have been reported.

PROCOAGUALANT FACTORS:

- Increased tissue factor
- increased von-wille brand factor, factor12a, factor 7a,
- increased plasminogen activator inhibitor.
- reduced tissue plasminogen activator.

- Abnormalities of platelets- Platelets can also be hyper aggregable due to increased plasma thromboglobulin and increased prostaglandin due to increased hepatic secretion as a part of

compensatory mechanism for hypoalbuminemia. Also decrease in platelet count in patients with chronic renal disease leads to prolonged bleeding time and altered haemostasis.[43]

- Abnormalities of clotting factors: Decreased protein c and antithrombin III as they are lost in urine, decreased fibrinolytic activity and increased concentration of factors II, V, VII, VIII, X, XIII due to increased hepatic synthesis as a part of compensation for hypoalbuminemia are the factors responsible for rendering a thrombotic state in patients with uremia. Thrombotic tendency is a known complication in patients on treatment with recombitant erythropoietin.

HEMATOLOGICAL ABNORMALITIES IN CKD AND THEIR CORELATION WITH VARIOUS ETIOLOGIES

ANEMIA AND DIABETES:

Anemia is a most common complication of diabetic nephropathy. It have been recognised that in diabetic individuals anemia is seen not only in advanced renal failure, but also frequently in patients with only minor derangements of renal function. At any level of GFR anemia is more frequent and severe in diabetic compared to nondiabetic patients.[45] anemia is a risk factor which neccesiates renal replacement therapy in diabetes . In addition, a lower Hb have been associated with a more

a rapid decline in the GFR and it is a significant negative predictor of end stage renal disease. Furthermore, treating anemia early in renal failure have been demonstrated to slow the rate of decline of renal function. Anemia also have a negative impact on patient's survival, and is considered to be an important cardiovascular risk factor associated with renal disease.[46]

Reasons for the earlier onset of anemia in patients with diabetes

- Severe symptomatic autonomic neuropathy, causing efferent sympathetic denervation of the kidney leading to loss of appropriate erythropoietin secretion.
- inhibition of EPO release. inappropriate response of erythropoietin to anaemia. interstitial fibrosis is associated with impaired function of EPO-producing fibroblasts and autonomic neuropathy is associated with a defect of "anemia-sensing" mechanisms and both may contribute to EPO deficiency.
- damage to the renal interstitium.
- systemic inflammation.
- Iron deficiency.
- Iatrogenic factors, e.g. ACE inhibitor treatment.[46]

HYPERTENSIVE NEPHROSCLEROSIS: Hypertension frequently accompanies advancing CKD, and it is often assumed as the cause rather than the effect of CKD. In fact, more patients develop hypertension from CKD than develop CKD from hypertension , ie,hypertensive nephrosclerosis. Hypertension exacerbates proteinuria and promotes tubulointerstitial inflammation, fibrosis, and tubular atrophy, further elevating BP. Some studies have shown that in ckd due to hypertension , anemia occurs only in very advanced stages.

AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE:

This is a common inherited kidney disorder. In this condition fluid-filled cysts develop in the kidneys. Patients become symptomatic later in life, usually between the ages of 30 and 40. However, symptoms can also start in childhood. PKD may impair kidney function and lead to kidney failure. PKD had higher levels of haemoglobin and EPO. Anemia is usually less common than in other types of chronic kidney disease, probably because erythropoietin production is preserved. This is true with initial stages, but as CKD progresses anemia ensues. PKD is characterized by numerous cysts. With age, the cysts will increase in both size and number.The large cysts will compress the adjacent kidney tissues and replace more healthy tissues,thus leading to progressive renal function decline. Healthy kidneys can produce erythropoietin (EPO)

which will stimulate the synthesis of red blood cells. However, the diseased kidneys in PKD fail to secrete enough EPO, thus resulting in anemia.

CHRONIC GLOMERULONEPHRITIS:

Focal glomerulosclerosis and membranoproliferative glomerulonephritis are the most likely chronic glomerulonephrities to progress quickly in adults. All hematological abnormalities have been noted in CKD due to chronic glomerulonephritis.

MATERIALS AND METHODS

General

Total no. of patients under study- 150 (50 in each stage 3 -5)

Period of Study:

Patients admitted as inpatients in the Department of Nephrology and medicine at Tirunelveli Medical College Hospital, during the period of August 2012 - October 2013 were included in this study of a prevalence of hematological abnormalities in various stages of CKD .

Geographic distribution:

Geographic distribution of the patients were predominantly from rural areas of Tirunelveli, Tenkasi, Tuticorin Districts.

The patients were selected randomly as per inclusion and exclusion criteria.

Inclusion criteria :

1. Patients with CKD (Stage 3-5)
2. Age > 14 years

Exclusion criteria :

1. Paediatric patients
2. Pregnant patients
3. Patients with known hematological disorder
4. H/O blood transfusion during last 3 months

5. Patients with ESRD treated with renal replacement in form of dialysis & renal transplantation.
6. Patients on drugs causing bone marrow suppression.

The details regarding age, sex, weight, monthly income the primary disease leading to CKD, tests used in diagnoses- ultrasound abdomen, blood urea, creatinine, urine- albumin sugar and were collected from the case charts.

Definitions:

For the purpose of the study, the following definitions were used.

1) Chronic kidney disease defined as the functional abnormality of the kidney manifested by elevated serum creatinine level of >1.5mg/dl for more than 3 months.
2) Anemia in CKD defined as Hb < 13g/dl in men and in women and <12g/dl.
3) The estimate of kidney function was obtained by assessing eGFR, using cockcroft-gualt equation.

Cockcroft-Gault equation

$$\text{Estimated creatinine clearance (ml/min)} = \frac{(140-\text{age}) \text{x body weight (kg)}}{72 \text{ x PCr (mg/dL)}}$$

Multiply by 0.85 for women.

Pcr – Serum Creatinine

INVESTIGATIONS

1. **FIRST LINE OF INVESTIGATIONS**:

TO ASSESS KIDNEY FUNTION:

- serum creatinine.
- blood urea.
- urine albumin.
- urine sugar.
- Ultrasound abdomen.

2. HEMATOLOGICAL INVESTIGATIONS:

- Hemoglobin.
- total count.
- differential count.
- RBC count, packed cell volume.
- MCV, MCH, MCHC.
- Platelet count.
- Bleeding time
- clotting time.
- Coagulation profile:-
    - prothrombin time.
    - activated partial thromboplastin time.
    - International normalized ratio.

- Peripheral smear study

LABORATORY METHODS :-

Serum creatinine and urea was done on clotted blood sample using an automated biochemical analyzer –XL-300 based on the principle of photometry. serum creatinine was estimated by modified jaffe method.

Hematological investigations-complete hemogram which includes total count, differential count (neutrophils,lymphocytes,eosinophils), Hb ,hematocrit, RBC count, MCV, MCH, MCHC, MCV, Platelet count, were done on EDTA (Ethylene diamine tetra acetic acid) blood sample on a standardized, quality controlled and maintained automated cell counter -Sysmex KX-21 which analyzes the physiological and chemical characteristics of blood cells based on the principles of electric resistance detecting method hydrodynamic focusing method and flow cytometry method using semiconductor laser.

Automated hematology analyzers use the cyanomethemoglobin method to measure the hemoglobin content of the red blood cell. In this method, hemoglobin is converted to cyanated methemoglobin (cyanmethemoglobin) by the addition of a Drabkin solution which contains potassium ferricyanide and potassium cyanide. Cyanated

methemoglobin maximally absorbs light at 540 nm, and the total amount of hemoglobin is determined by spectrophotometry. The hemoglobin concentration is measured in grams per deciliter (g/dL) of whole blood. The hematocrit is the ratio of the volume of red blood cells to the volume of whole blood. The automated hematology analyzer calculates the Hct from the RBC and MCV by the following formula:

Hct (%) = RBC (cells/L) x MCV

Because the Hct is a calculated value, it is less accurate than either the RBC or Hb, and is affected by errors in either or both of these measurements.

The MCV(mean corpuscular volume), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) are the red blood cell indices. The MCH is the concentration of hemoglobin per cell (hemoglobin mass/red blood cell), expressed in pictograms per cell (pg, 10-12 g). The MCH is calculated from the hemoglobin and RBC by the following formula:

$$\text{MCH (pg/cell)} = \frac{\text{Hb (g/L)}}{\text{RBC (cells/L)}}$$

The MCH is decreased in patients with anemia caused by impaired hemoglobin synthesis.

The MCHC is the average hemoglobin concentration per total red blood cell volume (ratio of hemoglobin mass to RBC volume), as determined from the following equation:

$$\text{MCHC (g/dL)} = \frac{\text{Hb (g/dL)}}{\text{Hct (L/L)}}$$

The MCHC is decreased in microcytic anemias where the decrease in hemoglobin mass exceeds the decrease in the size of the red blood cell. WBC is determined on EDTA-anticoagulated blood. RBCs are removed by lysis, and the total WBC is measured by electrical impedance or light scatter techniques. Nucleated red blood cells, unlysed red blood cells, platelet clumps, large platelets, and cryoglobulins may result in spurious WBC results. If these conditions are detected by the hematology analyzer, the specimen to be sent for a manual peripheral smear evaluation.

- Peripheral smear examination was done using Leishman's stain.
- Urine albumin was analysed by manual method using 3% sulfosaliclic acid.
- Urine sugar was analysed using benedict solution.
- Prothrombin time , activated partial thromboplastin time, INR was analysed in automated analyser sysmex CA-50.

- Serum protein was analysed in serum sample using automated analyser XL-300.

Analysis of the laboratory results

1. Estimated GFR levels were categorized into three stages.

STAGE	eGFR (ml/min/1.73m^2)
3	29-60
4	15-30
5	<15

2. Hemoglobin levels were categorized into three group:

GRADE	HB
MILD ANEMIA	10-12.9 for male, 10-11.9gm/dl for female
MODERATE ANEMIA	7-9.9 gm/dl
SEVERE ANEMIA	LESS THAN 7 gms/dl

3. Blood picture was categorized into:
    - normocytic normochromic,
    - microcytic hypochromic
    - normocytic hypochromic.

4. RBC count- males-4.5-6.5 million / cumm, females-3.8-5.8 million / cumm
5. Hematocrit:-males-40-54 females-37-47
6. Total count-4000-11000
7. prothrombin time:11-16secs
8. activated partial thromboplastin time:21- 26secs
9. INR:-1.00- 1.30
10. platelet count:-1,50,000-4,50,000
11. MCV:- 80-96 fl MCHC:- 32-36g/dl MCH:-27-32pg
12. bleeding time - 2.5-9.5 min
13. clotting time- 3-5 mts

<u>THE RESULTS WERE ANALYZED AS FOLLOWS:</u>

- 50 patients in each stage of CKD-3,4,5.
- The number and percentage of each variable with respect to 50 patients were estimated .
- RBC count, hemoglobin and peripheral smear, total count, differential count, platelet count, PT, Aptt, INR were compared and correlated with each other.

Statistical Analysis :

The details collected regarding all the selected patients were recorded in a master chart. Data analysis was done with the help of

computer by using SPSS software and Sigma Stat 3.5 version (2012). Using the software range, frequencies, percentage, mean, standard deviation and ‘p’ value were calculated through One way ANOVA, Chi square, Pearson and Spearman Correlation test and P value of < 0.05 has been taken as significant.

RESULTS AND OBSERVATION

Demography:

One hundred and fifty patients of CKD with a Egfr< 60ml/mt were included to study the prevalence of anemia and other hematological parameters.

There were 90 males and 60 females in this study. male predominance was observed. oldest male was a 74 years old. oldest female was 70 years. youngest female was 21 years . youngest male in this study was 25 years.

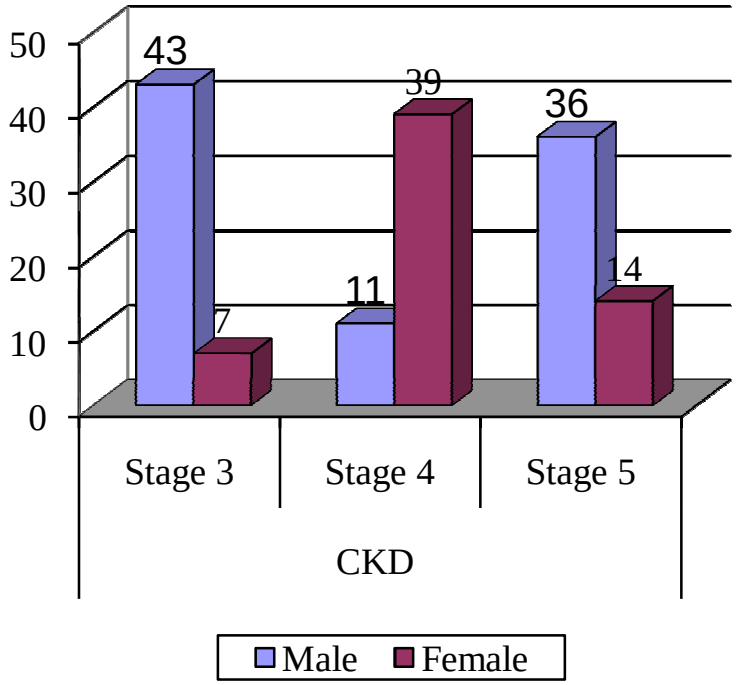
SEX DISTRIBUTION AND CKD STAGE CORRELATION

Sex		**CKD**		
		Stage 3	**Stage 4**	**Stage 5**
Male	90	43 (86%)	11 (22%)	36 (72%)
Female	60	7 (14%)	39 (78%)	14 (28%)
Total	150	50	50	50

Among 90 males 43 were in stage 3 ckd, 11 in stage 4, 36 in stage 5.

Among 60 females 7 were in stage 3, 39 in stage four, 14 were in stage 5.

Sex Distribution Vs CKD

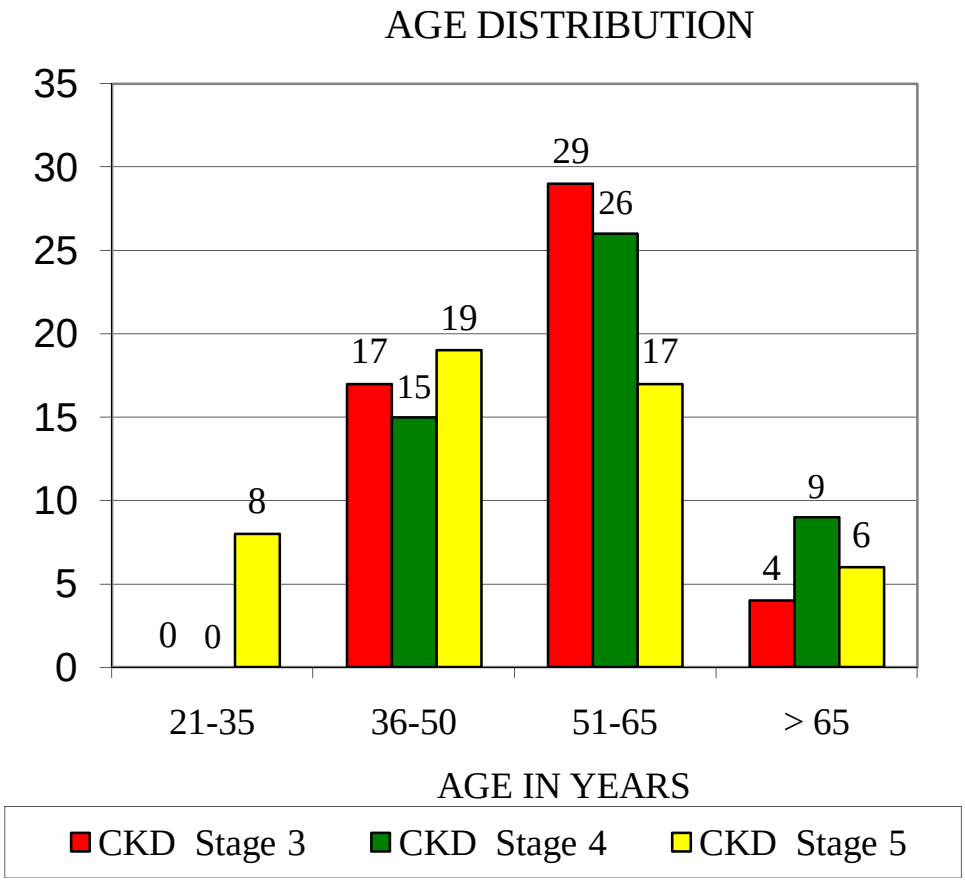


Above chart shows male predominance in stage 3 and 5 CKD. In stage 4 CKD female were predominant.

AGE DISTRIBUTION AND CKD STAGE CORRELATION

Age	**CKD**		
	Stage 3	**Stage 4**	**Stage 5**
21-35	0	0	8 (16%)
36-50	17 (34%)	15 (30%)	19 (38%)
51-65	29 (58%)	26 (52%)	17 (34%)
> 65	4 (8%)	9 (18%)	6 (12%)
Total	50	50	50

Maximum prevalence was observed in 51-65 yr age -group in stage 3 and 4. stage 5 ckd was prevalent in 36-50 age group. younger age individuals in the age- group of 21-35 tend to be more in stage 5 ckd. Individuals above 65 in this study was 19.among them 9 were in stage 4.six of them in stage 5. Four was in stage 3.



5.2 CLASSIFICATION BASED ON ETIOLOGY:

Etiology	CKD			
	Stage 3	**Stage 4**	**Stage 5**	**Total**
HTN	15	8	6	29(19.3%)
T2DM	12	10	8	30(20%)
HTN/T2DM	19	26	18	63(42%)
CGN(FSGS , MPGN, RPGN MGN, others)	3	6	17	26(17.3%)
ADPKD	1	0	1	2(1.3%)
Total	50	50	50	150 (100%)

Diabetes was the leading cause of CKD in this study followed by hypertension .63 patients had both hypertension and diabetes. 30 patients had only diabetes. so totally in 93 patients had diabetes was the underlying etiology.

In this study 29 patients had hypertension . In patients who presented with hypertension, it was either primary in etiology or secondary to chronic pyelonephritis. Among 29,eighteen patients was in stage 3. eight patients was stage 4 . only six was in stage 3…

Among the glomerular disease , membranoproliferative glomerulonephritis, rapid progressive glomerulonehritis, membranous nephropathy ,focal segmental glomerulonephritis were found to be the cause of ckd in 26 patients.

Autosomal dominant polycystic kidney disease was the etiologic factor in two patients(males). One patient was in stage 3 other was in stage 5.

ANEMIA IN CKD:

CORRELATION BETWEEN HEMOGLOBIN IN MALES AND CKD STAGES

Hb	**CKD**			
	Stage 3	**Stage 4**	**Stage 5**	**Total / p value**
<13g/dl No.of cases	16	9	36	61
mean	12.53	11.06	7.54	(67.8%) <0.001 sig
> 13g/dl	26	2	0	29

	Non Anemia			Mild (10 - 12.9)			Moderate (7.0 - 9.9)			Severe (<7)		
Male (90)	29			26			21			14		
CKD	3	4	5	3	4	5	3	4	5	3	4	5
	27	2	0	16	7	3	0	2	19	0	0	14

Among 90 males 61 patients had anemia. 26 were mildly anemic, 21 had moderate anemia, 14 were severely anemic. mean hb in stage 3 is 12.53. mean hb in stage4 was 11.06. mean hb in stage 5 was 7.54. it is evident that hb decreases as ckd stage progressed. this fall is statistically significant with p value <0.001.

CORRELATION BETWEEN ANEMIA IN FEMALES AND CKD STAGE

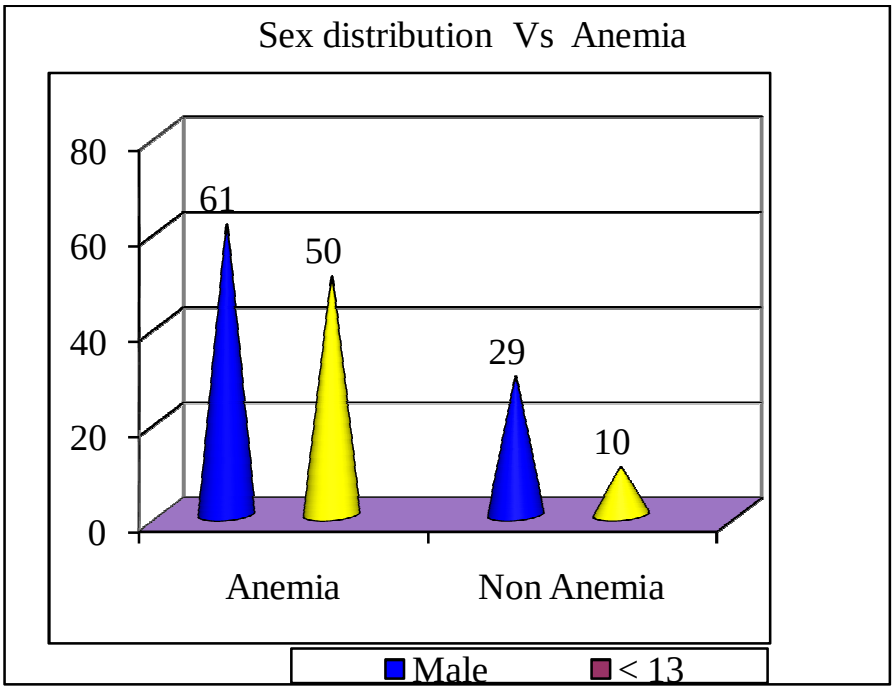
Hb	CKD			
	Stage 3	**Stage 4**	**Stage 5**	**Total**
< 12 No. of cases	6	30	14	50 (83.3%)
Mean	10.05	10.27	5.49	< 0.001 Sig
> 12 No.of cases	1	9	0	10 (16.7%)

	Non Anemia	Mild (10 - 11.9)	Moderate (7.0 - 9.9)	Severe (<7)

Female (60)	10			24			14			12		
CKD	3	4	5	3	4	5	3	4	5	3	4	5
	1	9	0	3	21	0	3	8	3	0	1	11

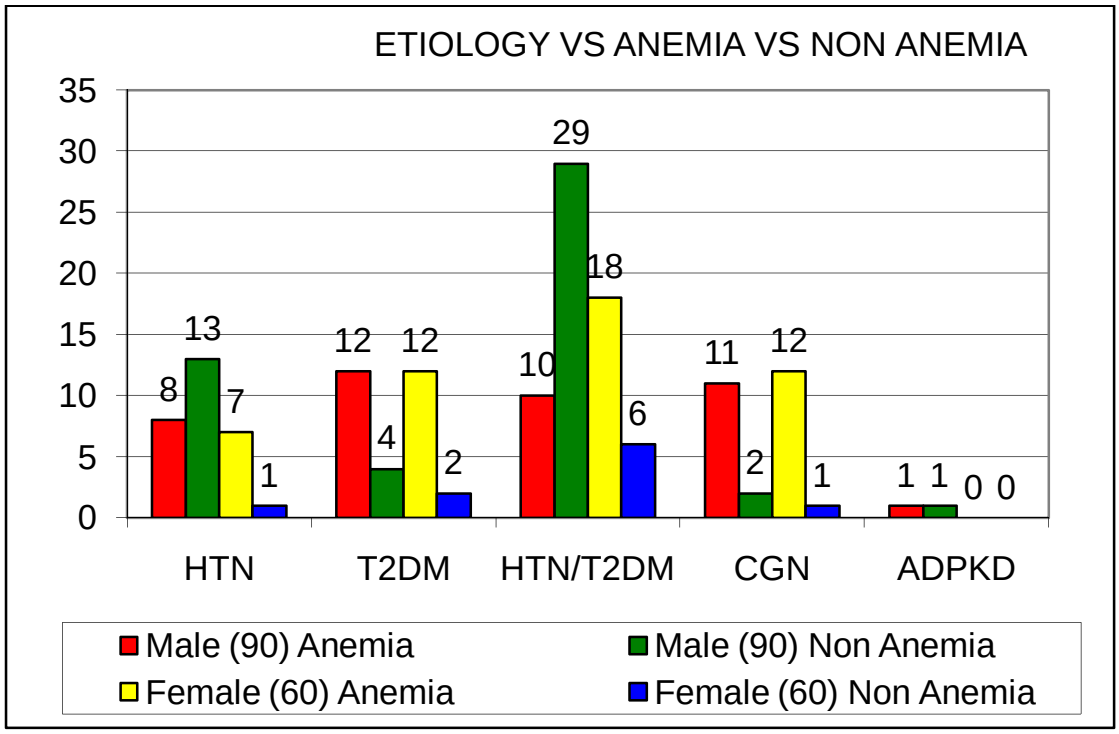
Among 60 females about 50 were anemic. 24 had mild anemia, 14 had moderate anemia, 12 had severe anemia.

Mean hb in stage 3 was 10.05. mean hb in stage 4 ckd was 10.49. mean hb in stage 5 was 5.49. so it was evident that anemia worsens as ckd progresses.this fall is statistically significant with p value <0.001.



Above figure shows that anemia is more prevalent in females.the reason may be generally in south Indian females anemia is highly prevalent,when they develop chronic kidney disease,which become an added factor, and so anemia is more prevalent in females.

CORRELATION BETWEEN ANEMIA IN CKD ITS VARIOUS ETIOLOGIES



In patients with ckd and due to hypertension, 8 male and 7 female had anemia.14(13 male,1 female) were not anemic.

- In patients were ckd was due to diabetes,12 male, and 12 female had anemia.

  6(4 male,2 female) was not anemic.

- In patients with both diabetes and hypertension,10 males and 18 females had anemia.26(20 male and 6 female) were not anemic.
- In patients with chronic glomerulonephritis, 11 males and 12 female had anemia. only 2 males and one female were not anemic.

Etiology	Total	**Male (90)**		**Female (60)**	
		Anemia	Non Anemia	Anemia	Non Anemia
HTN	29	8 (27.5%)	13(44.8%)	7(24.13%)	1(3.44%)
T2DM	30	12 (40%)	4(13.3%)	12(40%)	2(6.66%)
HTN/T2DM	63	10(15.8%)	29(46.03%)	18(28.57%)	6(9.52%)
CGN	26	11(42.3%)	2(7.69%)	12(46.15%)	1(3.84%)
ADPKD	2	1(50%)	1(50%)	0	0

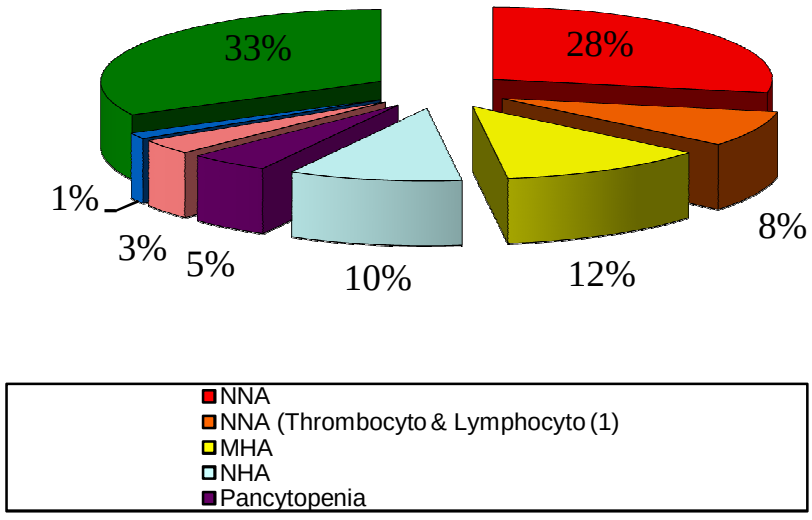
Anemia is highly prevalent in individuals, were diabetes and chronic glomerulonephritis were the underlying etiology for CKD. In hypertensive nephrosclerosis, percentage of females with anemia was more.

ADPKD WAS UNDERLYING ETIOLOGY IN 2 MALES:

CASE-1	**CASE-2**
• Mr.Akbar Ali.	Mr.Madasamy
• 53 Yr old male.	54 yr male.
• stage 3 CKD.	Stage 5 CKD.
• hb-14.2g/dl.	Hb-12.9g/dl.
• RBC count --4.21 lakhs/cumm.	RBC-2.24L/cumm.
• platelet count --3,21,000.	Platelet count-2,98,000
• Peripheral smear-normal picture	normal blood picture.

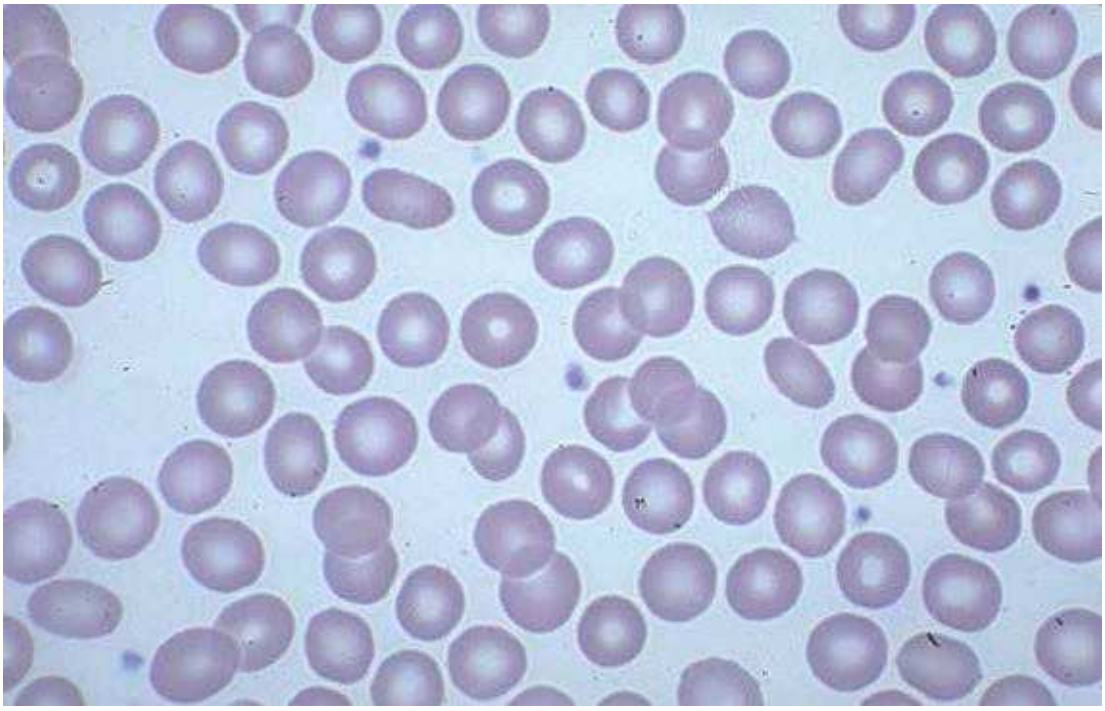
Among 2 males, one had hb just below cut-off value. otherwise all blood counts were normal.

PERIPHERAL SMEAR



NNA-normocytic normochromic anemia, MHA- microcytic hypochromic anemia, NHA-normocytic hypochromic anemia. In this study there was preponderance of normocytic normochromic anemia of 36%. Normal blood picture was seen in 33% . microcytic hypochromic picture was seen in 12%.next common picture was normocytic hypochromic which was 10%. Pancytopenia was seen in 5% of individuals.1% of individuals showed dimorphic blood picture.

Burr cells were present in 20 patients irrespective of peripheral smear picture and ckd stage.



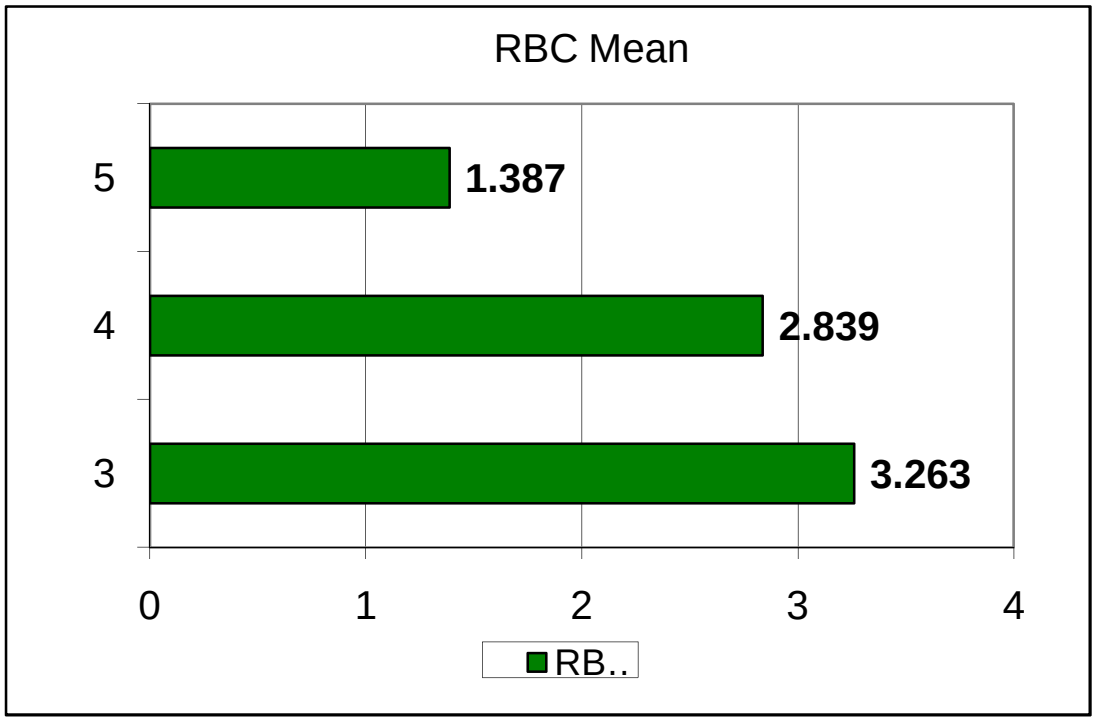
NORMOCYTIC NORMOCHROMIC BLOOD PICTURE

This is the most common type of blood picture seen in patients with anemia in ckd. here mean corpuscular volume is in

normal range (80-100). however hemoglobin and hematocrit is decreased. It is anemia of chronic disease.

Reticulocyte count was low < 0.5% in cases with anemia, indicating bone marrow suppression and EPO deficiency.

RBC COUNT IN CKD :



RBC count progressively decreased as ckd progressed from stage 3-5.

Stage	RBC Mean	SD	'p' value
3	3.263	0.734	< 0.001 Significant
4	2.839	0.847	
5	1.387	0.547	

RBC mean in stage 3 was 3.263 , with standard deviation- 0.734.

In stage 4 CKD, RBC mean was 2,839 , with SD- 0.847.

In stage 5 CKD,RBC mean was 1.387, with SD-0.547. Thus it is this study RBC count progressively decreased as stage declined. This fall was statistically significant with p value <0.001.

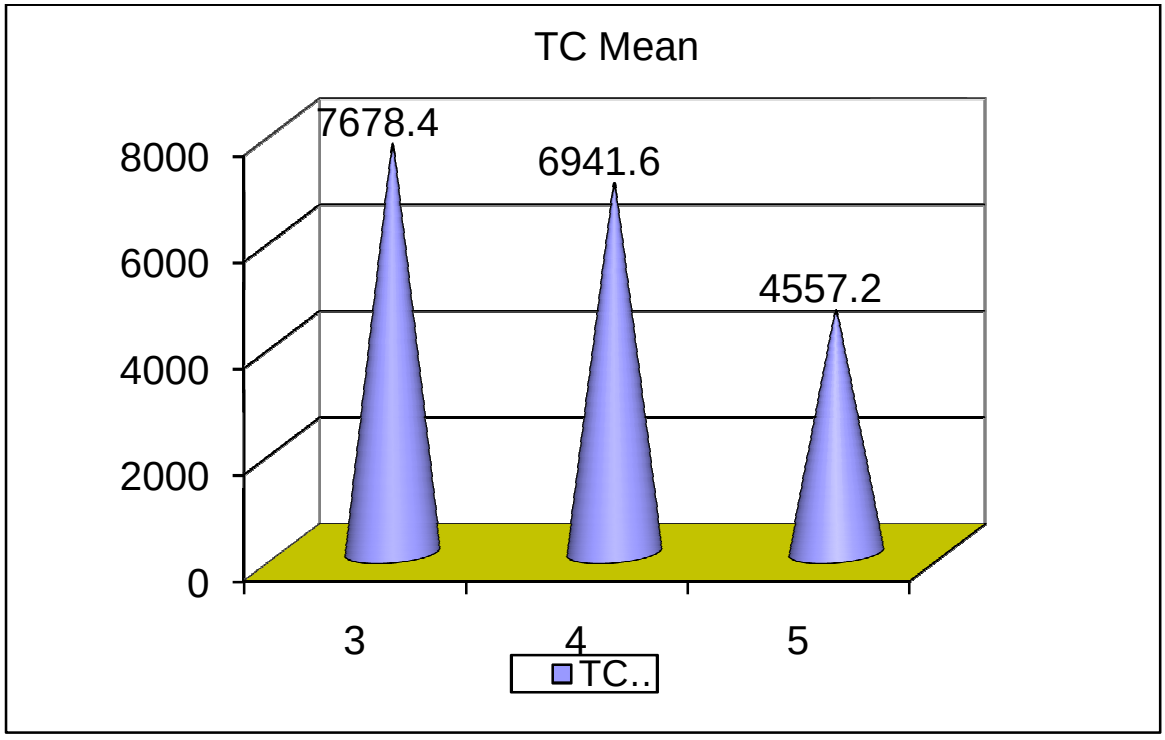
LEUKOCYTES IN CKD

In this study total count progressively decreased as CKD stage progressed. Quantitative as well as morphological changes were observed. The type of leukocytosis was neutrophilic which was 3%. In cases of neutrophilia, neutrophils with hypersegmented nuclei and coarse granulation of cytoplasm were common features. History of urinary tract infection was present in 2 of them. Eosinophilia was seen in six cases.

CORRELATION BETWEEN TOTAL COUNT AND CKD STAGE

Stage	TC Mean	SD	'p' value
3	7678.4	2425	< 0.001 Significant
4	6941.6	2621	
5	4557.2	3718	

Mean value of TC decreases stage by stage. The decreases are statistically significant ('p' value is < 0.001)

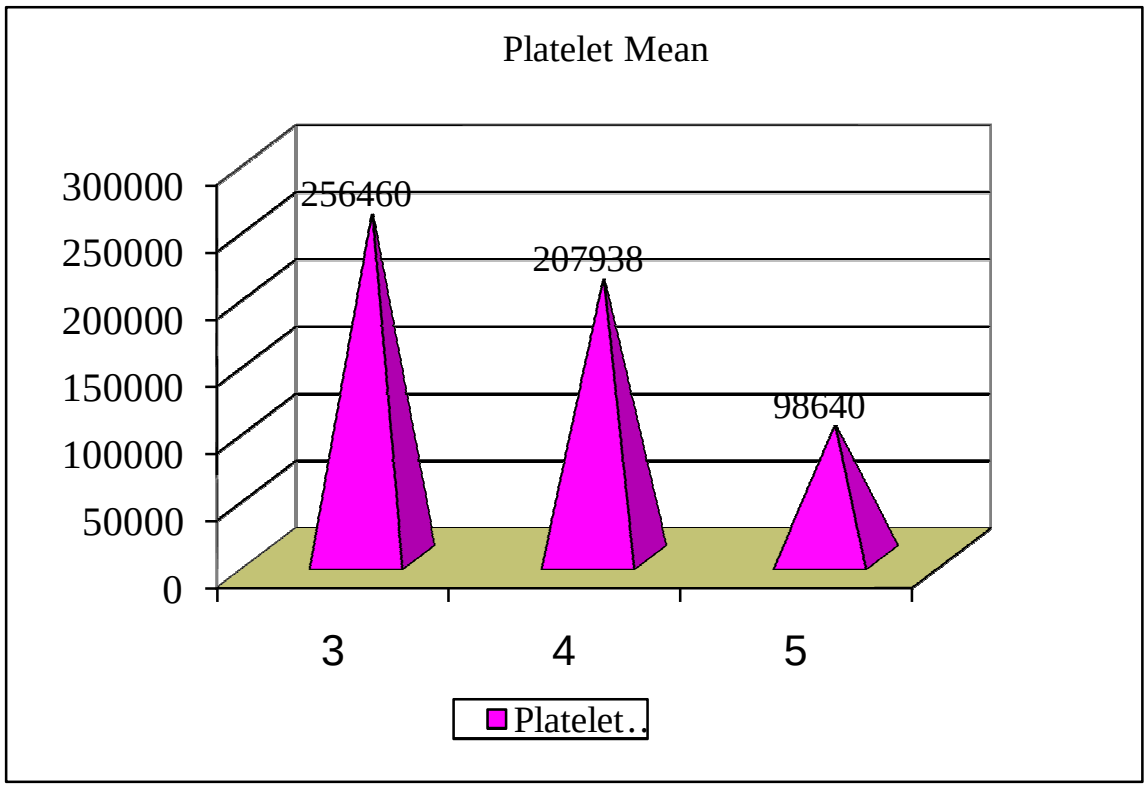


Mean TC In stage 3 CKD is-7678.4. in stage 4 CKD mean TC is-6941.6. in stage 5 CKD, mean was-4557.2.

PLATELETS' PROFILE IN CKD: platelet count progressively decreased as CKD stage declined.

PLATELET COUNT AND CKD STAGE CORRELATION

Platelet	Stage 3	Stage 4	Stage 5
< 1,00,000	0	1	35
100000 - 150000	2	15	5
150000 - 450000	48	34	10



CORRELATION BETWEEN PLATELET COUNT AND CKD STAGE

Stage	Platelet Mean	SD	'p' value
3	256460	57800	< 0.001 Significant
4	207938	72930	
5	98640	75472	

In stage 3 CKD, out of 50 patients, 48 had normal platelet count ranging between 1.5 lakhs and 4.5 lakhs. and 2 patients had mild decrease in platelet count ranging between 1 and 1.5 lakhs.

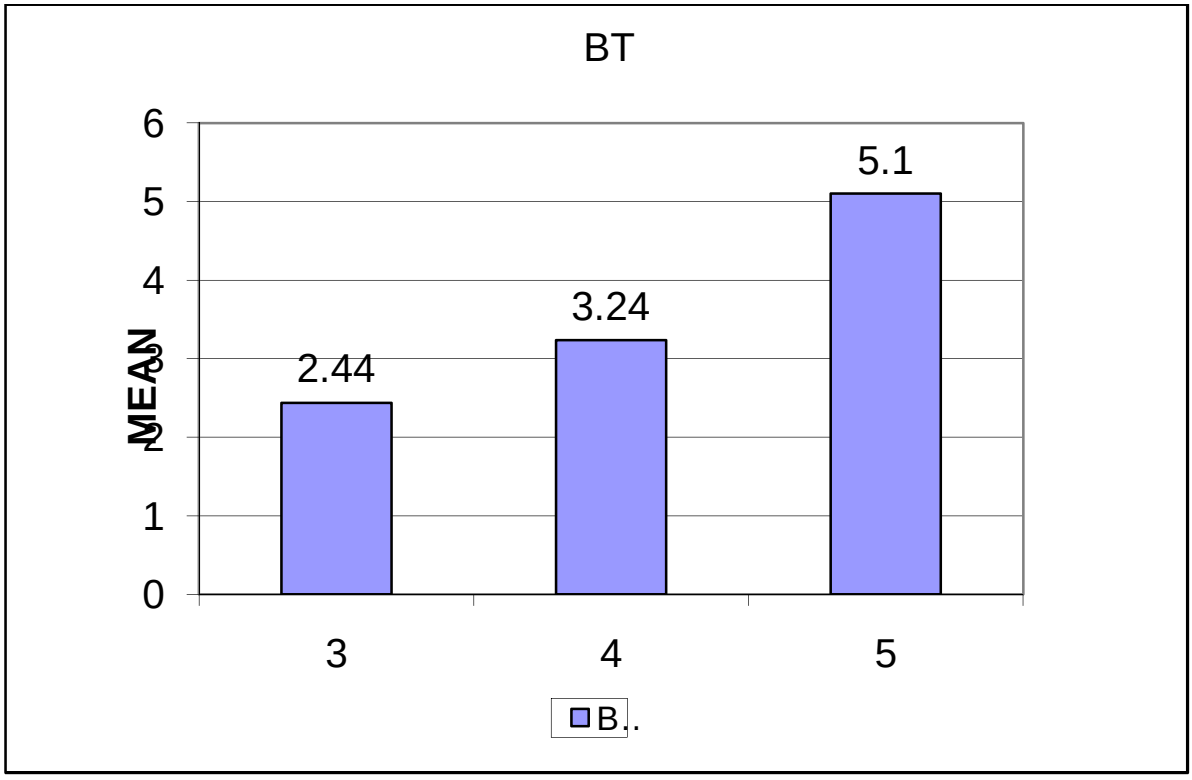
Among 50 patients in stage 4 CKD, 34 had normal platelet values,15 patients had mild decrease in platelet values ranging between 1 to 1.5 lakhs. only one had thrombocytopenia less than 1 lakh.

Among 50 patients in stage 5 CKD,10 patients had normal count.5 had mild decrease.but 35 patients had thrombocytopenia with platelet less than 1lakh.

This fall in platelet count as ckd stage declined was statiscally significant.

BLEEDING TIME

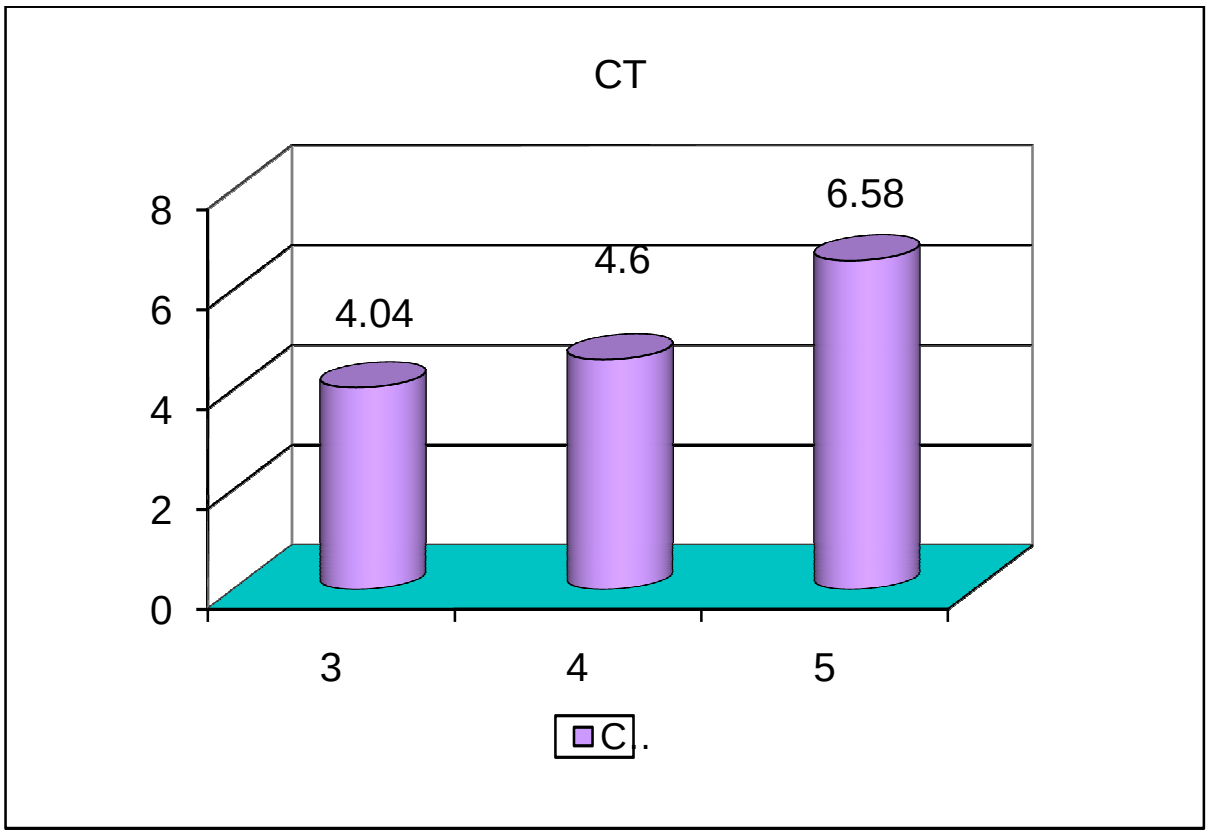
Stage	BT	SD	'p' value
3	2.440	1.37	< 0.001 Significant
4	3.240	1.88	
5	5.100	2.61	



Bleeding time prolonged as ckd progressed. Mean value increased stage by stage.increase was statistically significant (p value<0.001).normal bleeding time is 1-7 minutes.

CORRELATION BETWEEN CLOTTING TIME AND CKD STAGE

Stage	CT	SD	'p' value
3	4.04	1.29	< 0.001 Significant
4	4.60	1.94	
5	6.58	2.77	

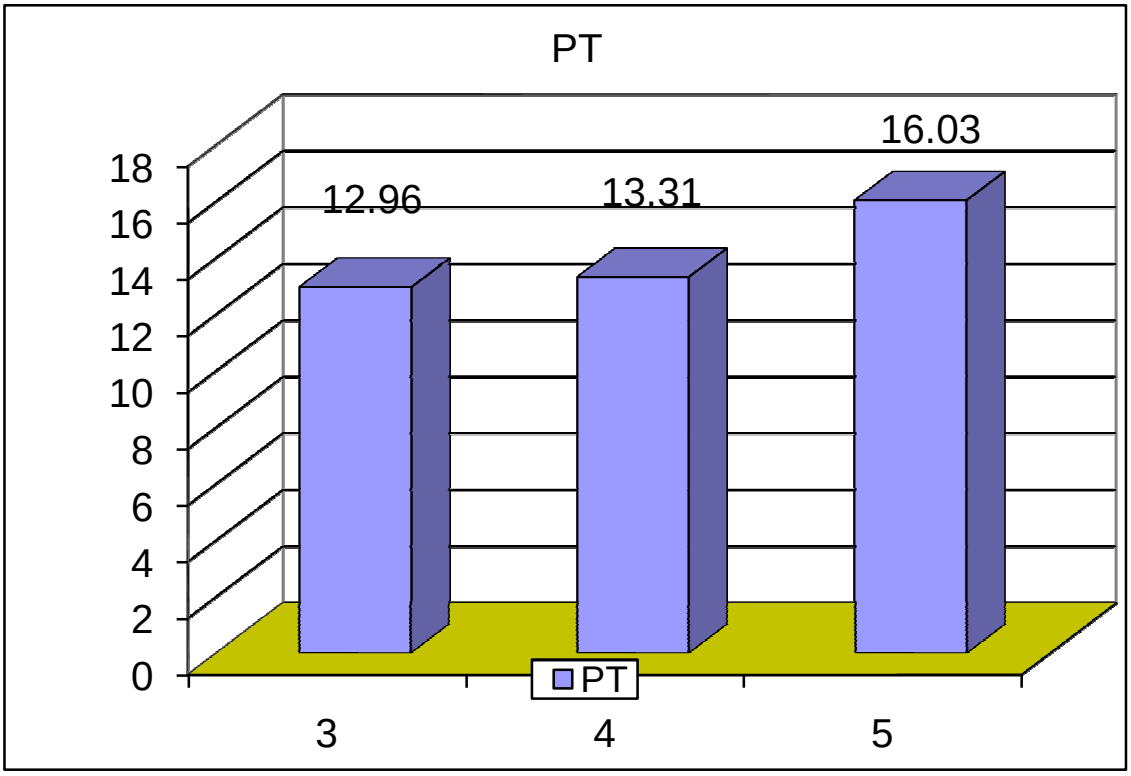


In study clotting time slightly prolonged as CKD progressed. Increase was statiscally significant. (p value-<0.001).

COAGUALATION PROFILE:

PROTHROMBIN TIME:

Stage	PT	SD	'p' value
3	12.96	1.18	< 0.001 Significant
4	13.31	1.49	
5	16.03	2.48	



Normal prothrombin time is 11-16 seconds. In this study it was found that prothrombin time tends to prolong as CKD stage declines in stage 3 CKD mean was 12.96. In stage 4 CKD, mean was 13.31 .in stage 5 CKD,mean was16.03. prolongation of PT was stastically significant.

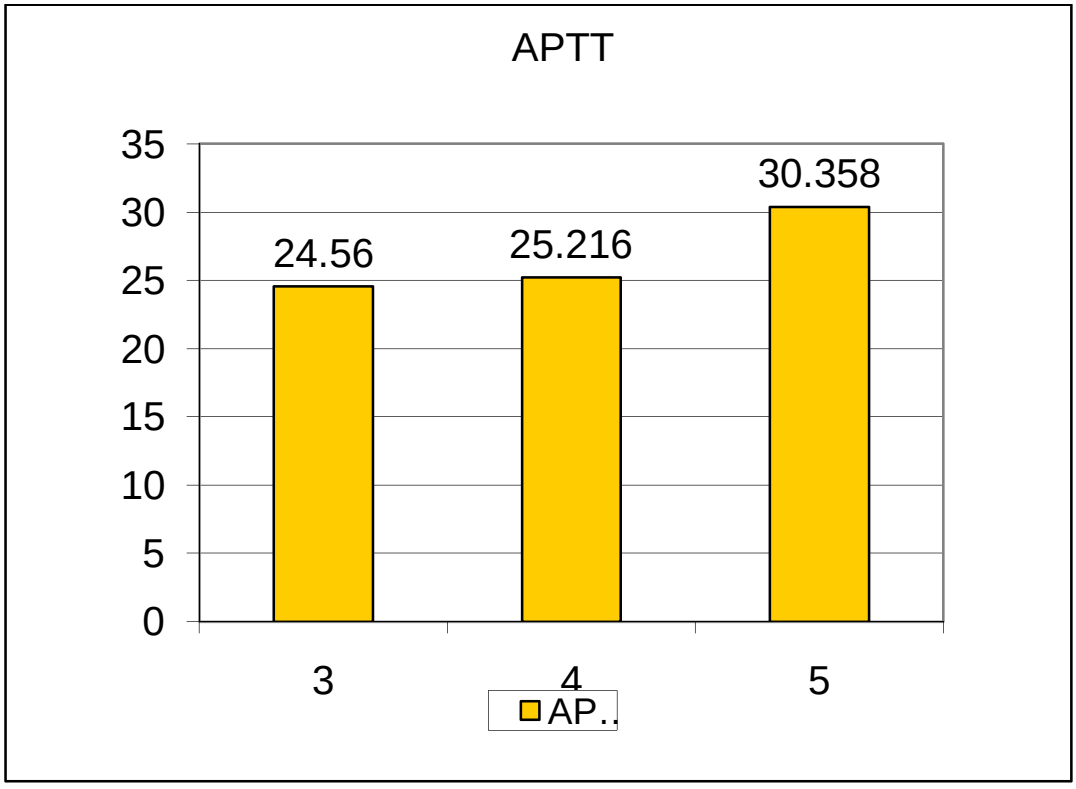
ACTIVATED PARTIAL THROMBOPLASTIN TIME:

APTT	Stage 3	Stage 4	Stage 5
< 21	0	0	3
21 – 28	46	46	11
> 28	4	4	36
Total	50	50	50

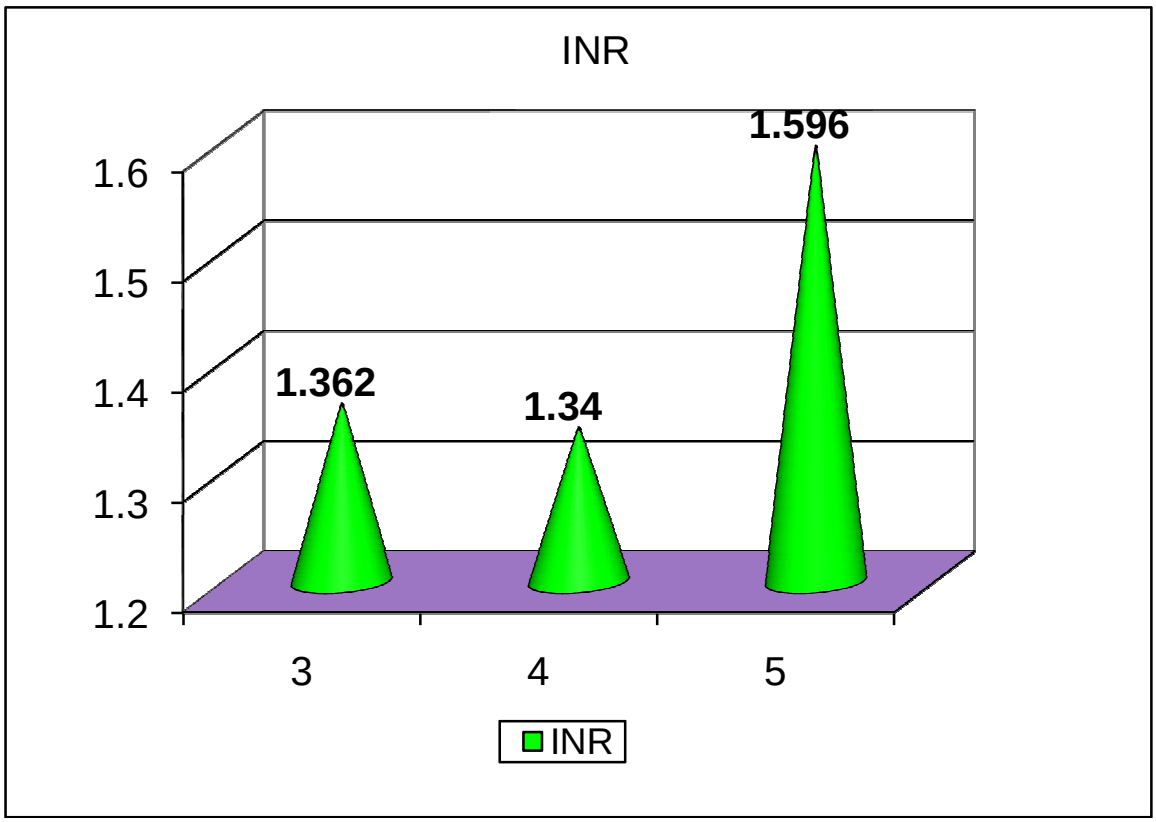
Stage	APTT	SD	‘p’ value
3	24.56	2.259	
4	25.216	2.646	< 0.001
5	30.358	5.567	Significant

In our study, APTT prolongs as CKD stage declines. From table is evident that 46 individuals in stage 3 and 4 ckd had normal APTT. Only four patients had prolonged APTT. But in stage 5 CKD thirty six patients had prolonged APTT. Mean APTT in stage 3 was 24.56.

In stage 4 mean APTT was 25.21. In stage 5 mean APTT was 30.35 prolongation was statiscally significant.(p value<0.001).



INTERNATIONAL NORMALISED RATIO:



In this study INR tends to prolong as ckd stage declined mean INR in stage 3 was1.362. mean INR in stage 4 was 1.34. mean INR in stage 5 was 1.596.

DISCUSSION

Prevalence and risk factors:

Chronic kidney disease has been a major public health problem and a major cause of morbidity and mortality worldwide. The actual prevalence of the initial stages of CKD is much more than the later stages.

Coresh J et al have reported a prevalence of 3.3%, 3.0%, 4.3%, 0.2% and 0.2% respectively in stages 1, 2, 3, 4, 5 of CKD [8].

However in clinical practice prevalence of stages 4 and 5 appears to be more because initial stages are asymptomatic and people present themselves when severity of symptoms increases.

This study included 150 patients with a diagnosis of CKD which was based on the estimated gfr levels. 50 in each stage3-5.

The prevalence is probably high compared to the western literature, as the patients seek treatment only at later stages when the severity of symptoms increases. Economic constraints also play a role.

Coresh J et al and Viswanathan et al have indicated that the prevalence of CKD is higher in older age groups with a male

preponderance. The former has also reported that 11% of individuals older than 65 years have CKD[8].

The present study also showed predominance in older age, the maximum number of cases in stage 3 and 4 was in the age group 51 - 65years. Cases in stage 5 was maximum in 36-50 age group. The older age and the male predominance can be explained by the underlying disease entity causing CKD. Diabetes and hypertension, both of which are etiological factors of CKD are prevalent more in older age group and male sex[8].

CKD is a progressive condition and its progression is influenced by the underlying kidney disease leading to CKD and the presence of risk factors[2].

Among the etiology of CKD , as per the DOQI advisory board guidelines, diabetes is the most common cause followed by hypertension, vascular diseases, glomerular diseases, cystic diseases and tubulointerstitial diseases[28].

Diabetes and hypertension are both etiological and risk factors. Comparison of the prevalence rates of etiologic disease of this study with that of DOQI guidelines has shown a similar trend,diabetes as leading

cause followed by hypertension, glomerular diseases and others. 63 cases had both diabetes and hypertension.

All hematological abnormalities were prevalent irrespective of the underlying etiology causing the CKD. Anemia was highly prevalent in patients , were diabetes and chronic glomerulonephritis were the underlying cause for CKD. In hypertensive nephrosclerosis, percentage of females with anemia was higher.

Hematological profile: Evaluation of anemia in chronic kidney disease begins with general clinical evaluation to assess both the possible causes and the clinical impact of anemia. Measures used to assess anemia and its causes include hemoglobin , hematocrit and peripheral smear.

Hemoglobin as an index of anemia Hemoglobin concentration is used as primary parameter because it can be directly measured for which there is an international standard and is not influenced by differences in technology. Hence this is a accepted parameter and so used as a baseline investigation in this study. In addition, for a patient to be repeated as and when required, this investigation is financially viable. The cut off value of the Hb level for the diagnosis of anemia in CKD is 13g/dl for males and 12g/dl for females as per WHO criteria and

further anemia has been graded as mild, moderate and severe anemia. KDOQI cut off values not taken into account since anemia is not graded in it.

In the present study, among males 67.8% had anemia. 83.3% of females had anemia. So it evident that females are more anemic than males. majority had mild anemia and very few had severe anemia.

But progressive decline in haemoglobin concentration, PCV and total red cell count was noted among our subjects as the disease progressed. This can be explained by a corresponding reduction in the synthesis and serum levels of erythropoietin which is a major drive for erythropoiesis in the bone marrow. Other factors may contribute to this in our subjects especially the associated malnutrition.

Hb levels decreased , indicating that the severity of anemia increased parallelly with the increase in the severity of CKD. The study done by Callen IR et al showed that anemia becomes more severe as CKD progresses[34].This is because, as CKD progresses, inhibition of bone marrow, deficiency of EPO, deficiency of iron and bleeding tendencies increase as a result of an increase in the circulating uremic toxins.

Normocytic normochromic blood picture was the most common in the study with 36%, followed by microcytic hypochromic which was 12%. then normocytic hypochromic picture which was 10%.This is also the scenario reported in the study by Callen IR et al [34] and in the study by the DOQI advisory board[33]

The MCV, MCH, MCHC of the patients with a normocytic picture were normal. RBC count also progressively decreased as CKD stage declined . reduced erythropoietin production is the principal factor responsible for the anemia in CKD. EPO deficiency leads to a decreased production of normal RBCs, the extent of which depends upon the extent of EPO deficiency.

Maximum Hb in study was 15g/dl. He was 54 yr old male, who had both diabetes and hypertension. In our study patient with Autosomal dominant polycystic kidney disease also had high Hb of 14.3g/dl. ADPKD is known to be associated with high Hb levels[4]. The patient was in stage 3 CKD. The patient was neither on dialysis nor on recombinant erythropoietin, which rules out the beneficiary effect of these. One of the patients had hypertension, which is also known to be associated with high Hb levels.

Microcytic hypochromic picture was seen in 12 % of cases. This is because of the presence of combination of factors leading to anemia. 5 patients had motion occult blood positive, which may be due to occult gastrointestinal bleeding. among them 4 had microcytic hypochromic anemia,0ne patient had normocytic hypochromic anemia. Associated iron deficiency would be the cause in them.

In patients with chronic kidney disease there are three primary disturbances that predispose to the development of iron deficiency as summarized below:

1. Increased external iron losses

   Blood retention by the hemodialysis filters and lines

   Frequent blood sampling for laboratory testing

   Occult gastrointestinal bleeding

   Accidental blood loss from the hemodialysis access

2. Sequestering of iron in storage tissues with decreased availability for erthropoiesis.
3. Decreased iron absorption Functional iron deficiency is a state in which iron is present in sufficient quantity in storage tissues with inefficient access to iron by erythroid precursors. In such a state there will be increased iron stores in bone marrow biopsy and associated increased serum ferritin levels. Given the chronic inflammatory state

in CKD, the increase in serum ferritin could be as an acute phase reactant. But transferrin saturation level will be abnormal indicating the low level of iron available for erythropoiesis.

Some of the peripheral smears showed elliptocytes, ovalocytes, and target cells. Seven patients showed pancytopenia.2 patients showed dimorphic anemia. 20 smears showed presence of burr cells. Burr cells are known to be associated with anemia in CKD. Burr cells also called as crenated cells which show an uneven distribution of spicules on their surface . Burr cells characteristically occur in uremia, where they represent damaged or fragmented red blood cell.

Abnormalities in white cells and platelets

Certain morphological and quantitative alterations were noted with leukocytes.40 patients showed leucopenia with total count less than 4000/cumm. In this study total count decreased as CKD stage declined. EPO is known to stimulate erythropoiesis with little effect on granulopoiesis[41]. A fall in the leucocyte count may be due to suppression of granulopoiesis because of erythropoietin deficiency. sequestration of neutrophils within the dialyser and in the lungs can also be an possibility, although studies with labeled cells show that they later return to circulation. . 5 patients in this study showed leukocytosis-reactive neutrophilia. 2 of them had clinical evidence of infection mainly UTI.

It is also possible that leukocytosis is a response to infection. It is possible that infections are due to impaired leukocyte function though they are increased in number [5].

In our study platelet count declined as progressed . EPO deficiency is a possibility as EPO is known to stimulate megakaryocytopoiesis to some extent[41].

HEMOSTATIC ABNORMALITIES:

In this study bleeding time and clotting time prolonged as ckd stage declined, indicating uremia is prone for abnormalities in hemostasis. Also prothrombin time , APTT, INR tends to prolong as ckd stage declined.

CONCLUSIONS

- CKD is prevalent in adult population with a male predominance in older age 51-65 groups.
- Diabetes and hypertension are the commonest etiological factors of CKD.
- Anemia is a common complication of CKD and the degree of anemia increases as CKD worsens.
- Mild anemia with normocytic normochromic picture with sparse distribution of RBCs is the most common picture.
- Anemia prevalence was higher in females.
- Etiological correlation - anemia and other hematological abnormalities were prevalent, irrespective of the underlying etiology causing the CKD.
- Leucophenia was observed, which worsened as CKD progressed.
- Thrombocytopenia is observed which worsened as CKD progressed.
- Both bleeding and thrombotic tendencies were observed as CKD worsened.
- Chronic kidney disease patients have lower haematological indices and the degree of changes depends on the severity of chronic kidney disease.

LIST OF ABBRVIATIONS USED (in alphabetical order)

APTT	Activated Partial Thromboplastin Time
BT	Bleeding Time
CKD	Chronic Kidney Disease
CGN	Chronic Glomerulonephritis
DOQI	Dialysis Outcome Quality Initiative
EGFR	Estimated Glomerular Filtration Rate
ESRD	End Stage Renal Disease
EPO	Erthropoeitin
FSGS	Focal Segmental Glomerulonephritis
GFR	Glomerular Filtration Rate
Hb	Hemoglobin
HCT	Hematocrit
HTN	Hypertension
INR	International Normalized Ratio
MDRD	Modification of Diet In Renal disease
MCV	Mean Corpuscular Volume
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MPGN	Membrano Proliferative Glomerulonephritis
MGN	Membranous Glomerulonephritis
SCR	Serum Creatinine

9. BIBLIOGRAPHY

1. National Kidney Foundation- DOQI Clinical Practice Guidelines on Chronic Kidney Disease. Executive summary. Am J Kidney Dis 2002; 39 (2) suppl 1: S1
2. European Renal Association - European Dialysis and Transplantation Association.European Best Practice Guidelines for the management of anemia in patients with CRF. When to begin the work-up of a patient for the diagnosis of anaemia. Nephrol Dial Transplant 1999; 14 (suppl 5):
3. Chandra M, Miller ME, Garcia JF, Mossey RT, McVicar M. Serum immunoreactive erythropoietin levels in patients with polycystic kidney disease as compared with other haemodialysis patients. Nephron 1985; 39(1): 26-2
4. Ruiz P, Gomez F, Schreiber AD. Impaired function of macrophage Fcγ receptors in end-stage renal disease. N Engl J Med 1990; 322 (11):717-722.
5. Minno GD, Martinez J, McKean ML, Rosa JDL, Burke JF, Murphy S. Platelet dysfunction in uremia- multifaceted defect partially corrected by dialysis. Am J Med 1985;79:552-559.
6. National Kidney Foundation- DOQI Clinical Practice Guidelines on Chronic Kidney Disease. Background. Am J Kidney Dis 2002; 39 (2) suppl 1: S32.

7. National Kidney Foundation- DOQI Clinical Practice Guidelines on Chronic Kidney Disease. Definition and stages of chronic kidney disease. Am J Kidney Dis 2002; 39 (2)suppl 1: S49.
8. National Kidney Foundation- DOQI Clinical Practice Guidelines on Chronic Kidney Disease. Definition and stages of chronic kidney disease. Am J Kidney Dis 2002; 39 (2) suppl 1: S56
9. National Kidney Foundation- DOQI Clinical Practice Guidelines on Chronic Kidney Disease. Chronic kidney disease as a public health problem. Am J Kidney Dis 2002; 39(1)suppl 1: S40.
10. National Kidney Foundation- DOQI Clinical Practice Guidelines on Chronic Kidney Disease. Definition and stages of chronic kidney disease. Am J Kidney Dis 2002; 39 (2)suppl 1: S48.
11. National Kidney Foundation- DOQI Clinical Practice Guidelines on Chronic Kidney Disease. Definition and stages of chronic kidney disease. Am J Kidney Dis 2002; 39 (2)suppl 1: S47.
12. National Kidney Foundation- DOQI Clinical Practice Guidelines on Chronic Kidney Disease. Executive summary. Am J Kidney Dis 2002; 39 (2) suppl 1: S24.
13. National Kidney Foundation- DOQI Clinical Practice Guidelines on Chronic Kidney Disease. Definition and stages of chronic kidney disease. Am J Kidney Dis 2002; 39 (2)suppl 1: S57.

14. National Kidney Foundation- DOQI Clinical Practice Guidelines on Chronic Kidney Disease. Definition and stages of chronic kidney disease. Am J Kidney Dis 2002; 39 (2)suppl1:S 58.
15. Coresh J, Astor BC, Greene T, Eknoyan G, Levey AS. Prevalence of chronic kidney disease and decreased kidney function in the adult US population: third national health and nutrition examination survey. Am J Kidney Dis 2003; 41 (1): 1-12
16. National Kidney Foundation- DOQI Clinical Practice Guidelines on Chronic Kidney Disease. Evaluation of laboratory measurements for clinical assessment of kidney disease. Am J Kidney Dis 2002; 39 (2) suppl 1: S88
17. National Kidney Foundation- DOQI Clinical Practice Guidelines on Chronic Kidney Disease. Evaluation of laboratory measurements for clinical assessment of kidney disease. Am J Kidney Dis 2002; 39 (2) suppl 1: S77.
18. National Kidney Foundation- DOQI Clinical Practice Guidelines on Chronic Kidney Disease. Evaluation of laboratory measurement
19. Jones CA, McQuillan GM, Kusek JW, et al. Serum creatinine levels in the US population: Third national health and nutrition examination survey. Am J Kidney Dis 1998;32 (6): 992-999.

20. Viswanathan V, Snehalatha C, Nair BM, Ramachandran A. Validation of a method to determine albumin excretion rate in type 2 diabetes mellitus. Indian J Nephrol 2003;
21. National Kidney Foundation- DOQI Clinical Practice Guidelines on Chronic Kidney Disease. Assessment of proteinuria. Am J Kidney Dis 2002; 39 (2) suppl 1: S99.s for clinical assessment of kidney disease. Am J Kidney Dis 2002; 39 (2) suppl 1: S79
22. National Kidney Foundation- DOQI Clinical Practice Guidelines on Chronic Kidney Disease. Definition and stages of chronic kidney disease. Am J Kidney Dis 2002; 39 (2) suppl 1: S51.
23. Harrisons principles of internal medicine 18th edition, Volume 2, Page No - 2337.
24. National Kidney Foundation- DOQI Clinical Practice Guidelines on Chronic Kidney Disease. Markers of chronic kidney disease other than proteinuria. Am J Kidney Dis 2002;39 (2) suppl 1: S104.
25. National Kidney Foundation- DOQI Clinical Practice Guidelines on Chronic Kidney Disease. Evaluation and treatment. Am J Kidney Dis 2002; 39 (2) suppl 1: S67.
26. McClellan WM, Knight DF, Karp H, Brown WW. Early detection and treatment of renal disease in hospitalized diabetic and hypertensive patients: Important differences between practice and published guidelines. Am J Kidney Dis 1997; 29 (3): 368-375

27. Viswanathan V. Prevention of diabetic nephropathy: A diabetologist's perspective.Indian J Nephrol 2004; 14:157-162.
28. Harrison textbook of internal medicine 18th edition, Volume 2, Page No - 2309
29. National Kidney Foundation- DOQI Clinical Practice Guidelines on Chronic Kidney Disease. Chronic kidney disease as a public health problem. Am J Kidney Dis 2002; 39(2)suppl 1: S39.
30. National Kidney Foundation- DOQI Clinical Practice Guidelines on Chronic Kidney Disease. Chronic kidney disease as a public health problem. Am J Kidney Dis 2002; 39suppl 1: S45.
31. Barry M.Brenner. Brenner and Rector s THE KIDNEY,8TH EDITION;VOL-2,chapter -49,page-1728.
32. Caro J, Brown S, Miller O, Murray T, Erslev AJ. Erythropoietin levels in uremic nephric and anephric patients. J Lab Clin Med 1979; 93(3): 448-458.87; 31: 72-76.
33. World Health Organization. Iron deficiency anemia: assessment, prevention and control;a guide for programmer manager.Geneva:WHO 2001. Accessed on 29th September, 2011 www.who.int/entity/nutrition/publications/.../GFF_References_en.pdf.

34. National Kidney Foundation- DOQI Clinical Practice Guidelines for the treatment of anemia of chronic renal failure. Introduction. Am J Kidney Dis 1997; 30 (4) suppl 3: S194.
35. Callen IR, Limarzi LR. Blood and bone marrow studies in renal disease. Am J Clin Pathol 1950; 20: 3-23
36. Segal GM, Stueve T, Adamson JW. Spermine and spermidine are non-specific inhibitors of in vitro hematopoiesis. Kidney Int 19
37. Manseh M, Grimes AJ. Red and white cell abnormalities in chronic renal failure. Br JHematol 1979; 42: 169-174.
38. Lim VS, DeGowin RL, Zavala D, et al. Recombinant human erythropoietin treatment in pre-dialysis patients. Ann Intern Med 1989; 110 (2): 108-114.
39. Rao DS, Shih MS, Mohini R. Effect of serum parathyroid hormone and bone marrow fibrosis on the response to erythropoietin in uremia. N Engl J Med 1993; 328(3):171-175.
40. Tarng DC, Huang TP, Chen TW, Fan CY, Chang JG. Resistance to recombinant human erythropoietin treatment in thalassaemic patients on chronic haemodialysis: a real clinical entity? Nephrol Dial Transplant 1996; 11: 1893-1895.
41. Aparicio M, Vincenceau P, Combe C, et al. Improvement of leucocytic Na- K pumpactivity in uremic patients on low protein diet. Kidney Int 1991; 40: 238-242.

42. Wieslander AP, Nordin MK, Kjellstrand PTT, Boberg UC. Toxicity of peritoneal dialysis fluids on cultured fibroblasts, L-929. Kidney Int 1991; 40: 77-79.
43. Hematological changes in chronic renal failure;international journal of scientific and research publications-volume 2,issue 9,sep-2012
44. coagualation and chronic kidney disease,DR.Amir KupermanM.D,thrombosis and hemostasis,pediatric homology.state of Israel-ministry of health.western galilee hospital-Nahariya.
45. Eur J Clin Invest. 2005 Dec;35 Suppl 3:66-74.Diabetic nephropathy and anaemia.Ritz E, Haxsen V.SourceDepartment Internal Medicine, Ruperto Carola University Heidelberg, Germany.

PROFORMA

NAME:

AGE: SEX:

OCCUPATION:

MONTHLY INCOME:

HISTORY OF DIABETUS MELLITUS:

HISTORY OF HYPERTENSION:

ETILOGY:

WEIGHT:

1) INVESTIGATIONS FOR ASSESSMENT OF RENAL FUNCTION:

A) BLOOD UREA:

B) SERUM CREATININE:

C) URINE ALBUMIN:

D) URINE SUGAR:

E) ULTRASOUND ABDOMEN:

2) INVESTIGATIONS FOR ASSESSMENT OF HEMATOLOGICAL CHANGES:

A)COMPLETE BLOOD COUNT:

Total count	Differential count	Hb	hematocrit	MCV	MCH	MCHC	Platelet count

B) MOTION OCCULT BLOOD:

C) SERUM ALBUMIN:

D) PERIPHERAL SMEAR STUDY:

E) RETICULOCYTE PERCENTAGE:

F) COAGULATION PROFILE:

BLEEDING TIME:

CLOTTING TIME:

	prothrombin time	Activated partial thromboplastin time
Control value Reference value		
TEST VALUE		

INTERNATIONAL NORMALISED RATIO:

ESTIMATED GLOMERULAR FILTRATION RATE :